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Studies on Royal Jelly Production by Honey Bees (*Apis mellifera* L.) in Himachal Pradesh, India

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Abstract: The investigations on royal jelly production by *Apis mellifera* are presented. The observations recorded on 12–24 h old larvae revealed the highest per cent acceptance during April (72.58) followed by May (70.02) and least during June (59.50). The royal jelly production recorded after 72 hours of grafting was more during mid May than in April or June. The average royal jelly production was significantly more in 10 frame colonies (329.50 mg/cup) than in colonies of 5 frame strength (253.30 mg/cup).

Keywords: Royal jelly, *Apis mellifera*, Colony strength

INTRODUCTION

Honey has since ages, been used in traditional medicines. But now-a-days other bee products are gaining importance all over the world due to their multifarious uses. Royal jelly is one of the valuable products of honey bees which has a good potential in clinical and therapeutic uses and also as a food supplement. This miraculous substance has been reported as a medicine against a number of diseases and disorders like anorexia, aging, wrinkles, obesity, arteriosclerosis, tumours etc. (Maly and Pacenovska, 1966; Tamura, *et al.* 1987,). Thus, this important product is in great demand all over the world. Asian countries like China and Taiwan are producing as much as 800 and 200 metric tonnes of royal jelly, respectively (Zhen-Ming, *et al.* 1992; Chang and Hsieh, 1993,). In India, no work has so far been done in this direction. Therefore, the present studies were undertaken on the royal jelly production in *Apis mellifera* colonies.

MATERIALS AND METHODS

Studies were carried out during April–June, 1994 in the apiary of Department of Entomology and Apiculture, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan. The per cent acceptance of 12 to 24 h old larvae and the royal jelly production were recorded using the mass queen rearing technique of Doolittle (1888)

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and Goyal (1987). For this purpose, 3 colonies, each of 5 and 10 frame strength were selected. Five more colonies were kept for the supply of larvae needed for grafting (breeder colonies), and supporting the above cell builder colonies.

The cell holders were made by fitting 3 bars at equal distances in a standard Langstroth frame. To each bar 10 queen cups were attached and thus there were 30 queen cups in each queen cell frame. Cell builder colonies were established 3 days before grafting. These colonies were dequeened and grafting was started after 3 days. It was ensured that colonies had frames of sealed brood, unsealed brood, pollen and honey. The comb with pollen was placed on one side of the cell building space and the young larvae on the other so that the nurse bees had to cross the queen cells to obtain pollen. The entire arrangement was re-established each week by providing sealed brood, unsealed brood and food stores from the reserved colonies.

Each queen cup was grafted with one larva of 12–24 h old and a batch of 30 such cells was given to each colony. Observations were made on per cent larval acceptance after 24 hours of grafting, during April to June, 1994. The royal jelly was harvested from these cells after 72 hours and weighed in an electric balance (Dhona Type).

RESULTS AND DISCUSSION

The observations recorded on the percent acceptance of larvae during April–June, 1994 (Table 1) revealed that there was no significant difference in the acceptance of larvae between 5 and 10 frame strength colonies. The average cell acceptance was 66.62 and 68.11 per cent in 5 and 10 frame colonies, respectively. A larval acceptance between 24.30 and 92.90 per cent has been reported by different workers (Okada and Obata, 1962; Snelgrove, 1966; Macicka, 1985; VanToor and Littlejohn, 1994) and the present findings are well within the range reported by these workers.

Table 1 Mean per cent acceptance of larvae after 24 hours of larval (12–24 h old) grafting during April–June, 1994

Month of observation	Per cent acceptance Colony strength (frame) 5	10	Mean
April	71.25(57.61)*	73.92(59.37)	72.58(58.49)
May	69.50(56.52)	70.54(57.19)	70.02(56.85)
June	59.12(50.30)	59.87(50.74)	59.50(50.52)
Mean	66.62(54.81)	68.11(55.77)	

$CD_{(0.05)}$ Month = (1.25)
Frame = (*NS*)
Month × Frame = (1.76)

*Figures in parentheses are arc sin transformed values

The acceptance of grafted larvae in the present study varied significantly during different months. The average cell acceptance was 72.58, 70.02 and 59.50 per cent during April, May and June, respectively. The higher acceptance of larvae during

April was probably due to the presence of sufficient amount of bee flora and swarming instinct in the colonies than during May and June. Floral plants particularly the pollen sources declined during June for honey bees, which significantly affected the acceptance of larvae, since availability of pollen in abundance is a pre-requisite in royal jelly production.

Table 2 Amount of royal jelly produced (mg/cup) after 72 hours of larval (12–24 h old) grafting

Date of observation	Royal jelly production (mg/cup) Colony strength (frame) 5	10	Mean
20.04.1994	248.15	321.53	284.80
12.05.1994	257.85	329.17	293.50
15.05.1994	253.58	340.45	297.00
18.05.1994	250.81	328.86	289.80
31.05.1994	253.12	328.07	290.60
02.06.1994	256.18	328.91	292.50
Mean	253.30	329.50	

$CD_{(0.05)}$ Month = 5.50
Frame = 3.17
Month × Frame = 7.79

The amount of royal jelly produced after 72 hours of grafting varied to some extent during different dates of harvest (Table 2). Irrespective of strength of the colonies, the minimum amount of 284.80 mg/cup was harvested during April and maximum of 297.00 mg/cup during mid May. The royal jelly production was significantly more in 10 frame colonies during different dates of observations as compared to the 5 frame colonies. This amount ranged between 248.15 to 257.85 mg/cup in 5 frame colonies as compared to 321.53 to 340.45 mg/cup, in 10 frame colonies. Chang and Hsieh (1993) have found that the royal jelly production increased with increase in the colony size. In the present study too, we found that on an average 5 frame colonies produced less royal jelly (253.30 mg/cup) than 10 frame colonies (329.50 mg/cup). The royal jelly production per cup has been reported to vary between 200 to 421.94 mg/cup after 72 hours of grafting by different workers (Okada and Obata, 1962; Root, 1966; Ono, 1982; Brown, 1989; Chang and Hsieh, 1993; VanToor and Littlejohn, 1994).

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Preliminary Investigations on *Lasioptera* sp. (Diptera: Cecidomyiidae) inducing galls on the leaves of *Dioscorea* spp. in Nigeria

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Abstract: Preliminary investigations were conducted on *Lasioptera* sp. inducing galls on the leaves of *Dioscorea* spp in Nigeria. From 5-10% of *D. rotundata* and *D. alata* leaves were galled. Gall shapes varied, with many being amorphous. Galling defaces the leaves and also prevents photosynthesis on the parts of the leaf with galls. *Lasioptera* also induced galls on the leaves of a tuberous weed species, *Cacina trichantha* Oliv. (Icacinaceae). Infestation of yams usually started in April and peaked in June/July. Parasitisation of *Lasioptera* by *Omphale* sp. in both the yams and the weed hosts of *Lasioptera* was established.

Keywords: *Lasioptera* sp, Cecidomyiidae, galls, *Dioscorea*.

INTRODUCTION

In Nigeria two categories of insect pests (direct/indirect) damage cultivated yams (*Dioscorea* spp.) in field plots. The more important ones are the direct pests which include the yam beetles, *Heteroligus* and *Prionoryctes* spp. (Coleoptera: Dynastidae) (Taylor, 1964), scale insects, *Aspidiella* sp. (Homoptera: Diaspididae) (Nwana, 1977), mealybugs, *Pseudococcus* spp. (Homoptera: Pseudococcidae) (Akinlosotu, 1984) and field crickets (Gryllidae). Their total effect results in low root yield and reduced revenue. The indirect insect pests also include the field crickets (Gryllidae) which cut vines (Jerath, 1965) and some Chrysomelids (Jerath, 1965; Akinlosotu, 1985; Echendu and Emehute, 1992). The activities of these indirect pests on yam minisett plots cause loss of established stands as the cutting and/or defoliation of tender vines affect plant vigour and total plant development.

Recently galled leaves of *D. rotundata* Poir and *D. alata* L. cultivars were observed in Nigeria. The galling phenomenon is now a frequent occurrence in yam plots. Gall insects such as the Hessian fly and rice gall midge are known important agricultural pests. As there is no available literature on galling on yam leaves, this study was carried out in 1991 and 1992 to obtain information on the identity and some aspects of the ecology of the causal agent, as well as the nature of plant damage.

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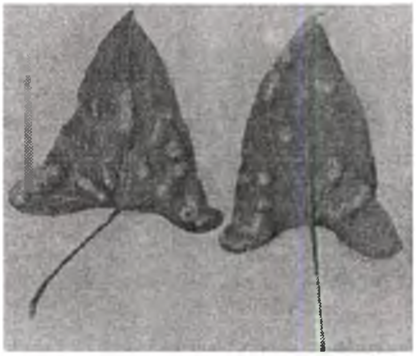

Fig. 1. Galled leaf of *D. alata* cv. Um 680.

Fig. 2. Galled leaf of *Icacina trichantha*

MATERIALS AND METHODS

Collection of galled leaves and identification of insect species: Galled leaves (Fig. 1) were obtained from field plots of *D. rotundata* and *D. alata* plants. The excised galls (0.7×0.4×0.3 cm) were held in open laboratory conditions at 25.0 - 30.0° C and a relative humidity (RH) 65.0-85.0% in well aerated petridishes lined with moistened white filter paper to prevent gall desiccation and the drowning of insect spp. emerging from the galls. Each petridish held one excised gall. The number and spp. of insects that emerged from each gall as well as their sequence of emergence were recorded.

Damage: Assessment of damage was based on counting galled/ungalled leaves/infested plant (200 plants) and testing for the presence or absence of starch in 50 galled leaves using the method described by Vines and Rees (1959).

Ecological studies: Field visits to relate galling with the phenology of the crop were made to establish the seasonal occurrence, alternative host(s) and the natural enemies of the causal agent.

RESULTS

Emergence and identification of insect species: Two insect spp. emerged from the galls. One was *Lasioptera* sp. (Diptera; Cecidomyiidae) the causal agent for gall formation; the other was *Omphale* sp. (Hymenoptera: Eulophidae) parasitic in *Lasioptera* sp. The sequence of emergence was *Lasioptera* sp. followed by *Omphale* sp. 3-4 days after. In both species emergence was completed within two days of commencement. Mean adult emergence for *Lasioptera*/gall size used was 17 and 25 in *D. rotundata* and *D. alata*, respectively; while for *Omphale* it was 21 and 20 in *D. rotundata* and *D. alata*, respectively (Table 1). Individuals of *Lasioptera* emerged more where less *Omphale* adults emerged. In some cases only *Lasioptera* or *Omphale* individuals emerged from the galls. The per cent incidence of *Lasioptera* and *Omphale* in the sampled galls (n = 40) is shown in Table 2. Freshly emerged adults of both spp. held in captivity without food in the open laboratory died within three days of emergence.

Table 1. Number of individuals of *Lasioptera* and *Omphale* spp. that emerged from 20 sampled galls each from *D. rotundata* and *D. alata* leaves

D. rotundata			*D. alata*		
Galls S/No.	Lasioptera (Host)	Omphale (parasitoid)	Galls S/No.	Lasioptera (host)	Omphale (parasitoid)
1.	10.0	22.0	1.	26.0	16.0
2.	31.0	2.0	2.	1.0	29.0
3.	9.0	13.0	3.	8.0	24.0
4.	7.0	17.0	4.	38.0	11.0
5.	41.0	34.0	5.	1.0	26.0
6.	7.0	17.0	6.	27.0	11.0
7.	41.0	12.0	7.	12.0	20.0
8.	14.0	15.0	8.	19.0	52.0
9.	76.0	22.0	9.	89.0	30.0
10.	3.0	13.0	10.	20.0	13.0
11.	6.0	46.0	11.	94.0	36.0
12.	5.0	43.0	12.	7.0	37.0
13.	6.0	14.0	13.	36.0	0.0
14.	8.0	24.0	14.	18.0	0.0
15.	16.0	24.0	15.	10.0	20.0
16.	4.0	33.0	16.	0.0	28.0
17.	0.0	29.0	17.	15.0	10.0
18.	0.0	20.0	18.	20.0	8.0
19.	22.0	10.0	19.	10.0	20.0
20	23.0	0.0	20.	48.0	0.0
$\bar{x}$	16.5	20.5	$\bar{x}$	25.0	19.5

Table 2. Incidence (%) of *Lasioptera* and *Omphale* spp. in sampled galls ($n = 40$) of leaves of *Dioscorea* spp.

No. of galled leaves	*Lasioptera*	*Omphale*
36	90.0	90.0
4	10.0	0.0
3	0.0	7.5

Damage: Galls were more prominent on the lower (abaxial) surface of galled leaves, but insect emergence was from the upper (adaxial) surface. Part of the puparial case of *Lasioptera* remained in the gall while the remaining part stuck out on the surface of the gall. A count of galled/ungalled leaves in randomly selected plants, 20 each of *D. rotundata* and *D. alata*, revealed 5.0-10% infestation with some leaves carrying more galls than others. Galls which assumed varied shapes and some amorphous did not cause premature leaf fall. However galled areas or portion of infested leaves gave

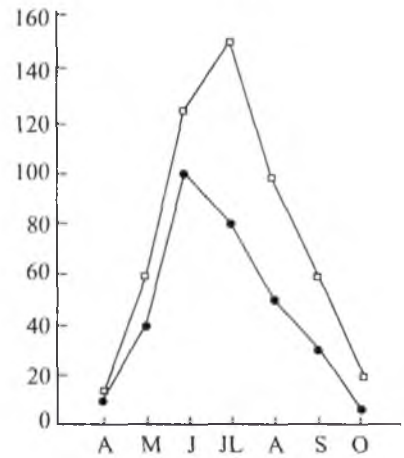

Fig. 3. Mean monthly variations on galling incidence on *D. rotundata* (●—●) and *D. alata* (□—□) 1991 and 1992

negative result when the test for starch was performed on the infested leaves.

Ecological studies: Galling commenced early April, peaked in June/July and was lowest in October (Fig. 3) when only *D. alata* had fresh galls. During the off season of yams in the field, *Lasioptera* tided over this period galling on the leaves of a wild tuberous herb, *Icacina trichantha* Oliv. (Icacinaceae) (Fig. 2). *Omphale* individuals also parasitised *Lasioptera* in *I. trichantha*, where galling occurred throughout the year.

DISCUSSION

Lasioptera sp. has not been reported before as a pest of yam in Nigeria and probably elsewhere in the world. This preliminary study revealed that it could reduce root tuber yield as galling, which peaked during the active growth period of yam (June/July) (Fig. 3) in Nigeria, prevented photosynthesis which affects the amount of photosynthetic sink for storage root initiation and bulking. The degree of damage by *Lasioptera* may be seriously limited by *Omphale* which successfully parasitised *Lasioptera* in 90.0% (36) of the sampled 40 galls (Table 2). Both *Lasioptera* and *Omphale* emerged from 36 galls, equivalent to 90.0% of the sampled 40 galls; *Lasioptera* only emerged from 4 galls, equivalent to 10.0%, while *Omphale* only emerged from 3 galls, equivalent to 7.5%, of the sampled 40 galls. It is therefore inferred that only the *Lasioptera* in 10.0% of the sampled galls escaped parasitisation by *Omphale* while the *Lasioptera* in 7.5% of the sampled galls were completely and successfully parasitised by *Omphale* thus preventing *Lasioptera* development and emergence.

Lasioptera and its parasitoid, *Omphale*, thrive in *D. rotundata* and *D. alata* judging from the mean emergence of the two insect species (Table 1). These observations indicate a strong coevolutionary host-parasitoid link existing between *Lasioptera* and *Omphale* with the host plants and further underline what might happen if *Omphale* is handicapped or eliminated as would likely happen where contract insecticides are

used to control other pests of yam such as the yam leaf beetles (Crioceridae) (Theberge, 1985). Since the galled parts of the infested leaves tested negative for starch, it is envisaged that heavily galled yam plants would give low tuber yields due to the reduced photosynthetic activities. Low tuber yield was actually observed at Mbaise in Imo State, Nigeria in a farmer's yam plot that was severely galled in 1993. From the preliminary study *Lasioptera* is a pest capable of causing yield reduction. The parasitoid, *Omphale*, and possibly other environmental factors are limiting the full manifestation of the effect of *Lasioptera* on yams. Future research efforts are aimed at detailed bionomics of *Lasioptera* and *Omphale* in order to develop a sound control strategy against *Lasioptera*. Such control strategy would incorporate the elimination of the all season alternative host plant(s) such as *I. trichantha* since yams are seasonal crops.

ACKNOWLEDGEMENTS

I sincerely thank the Director and staff of the International Institute of Entomology, London, for identifying *Lasioptera* and *Omphale* species. Dr. S. E. Okeke of Imo State University, Nigeria kindly identified *I. trichantha*. The provision of facilities for the study by the Director, National Root Crops Research Institute, Umudike, Nigeria, is highly appreciated.

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Electrophoretic and Quantitative Changes in the Protein Content of *Lipaphis erysimi* (Kalt.) Under the Influence of Methoprene Treatment

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Abstract: The effects of three sublethal doses (64, 320 and 1,600 ppm) of methoprene administered for 48 hours, was studied on all the four nymphal instars and adults of *L. erysimi*. Methoprene was found to influence the protein content significantly both quantitatively and electrophoretically as the total protein content as well as the number of bands increased with the treatment in all the developmental stages and adults.

Keywords: Methoprene, Proteins, *Lipaphis erysimi*, development.

INTRODUCTION

Methoprene (juvenile hormone analogue) is among the IGRs which have gained recognition in the IPM programmes of a number of insect pests of crops, as well as for insects of veterinary and medical importance. A wide range of species specific morphogenetic influences have been manifested by it, either by interfering with the titres of endogenous juvenile hormones or by having direct toxic effects (Staal, 1975). In spite of the fact that a large number of reports about its morphogenetic activity on various insect species, are available (Takahashi and Ohtaki, 1975), the information about its influence on various biochemical entities which form the basis for morphogenetic effect, is almost lacking (Himeno, 1979; Maa, 1987).

Proteins constitute the basic entities in the living being and undergo both quantitative changes during development (Engelmann, 1979). Therefore, it was envisaged to analyze quantitatively and electrophoretically the effects of methoprene on the total body proteins of mustard aphid during its development. The mustard aphid, *Lipaphis erysimi* (Kalt.) is an important pest of *Brassica* crops, which has already been reported to get adversely influenced both at morphogenetic level and phasephenism when treated with methoprene (Rup and Gill, 1993).

MATERIALS AND METHODS

L. erysimi was reared on radish (*Raphanus sativus Linn.*) sown in 1 Kg capacity earthen pots. About 150 virginoparae were released on uninfested plants to lay their

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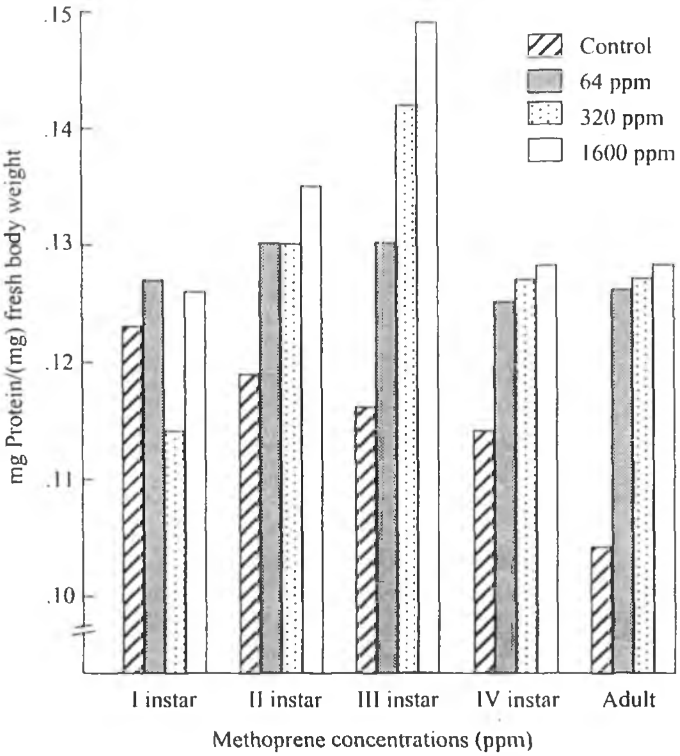

Fig. 1. Protein content of *L. erysimi* after treatment with methoprene.

progeny for 6 hours and then the second, third, fourth instar nymphs and adults were collected after 48, 102, 158 and 218 hours, respectively. In experiments with Ist instar nymphs, 30 females per replication were released directly on the treated plants for 6 hours for laying the nymphs.

Three concentrations of methoprene (64, 320 and 1,600 ppm and acetone as control) were sprayed with the help of hand atomizer on both the surfaces of the young leaves (3–4) of one month old plants. The aphids (nymphs or adults) were fed upon the treated leaves for an interval of 48 hours before the quantitative and electrophoretic analyses for proteins were done.

Fifty mg of fresh weight aphids/nymphs were homogenized in 2 ml of 0.1 M phosphate buffer prepared in 0.85% NaCl for the extraction of proteins, according to the method advocated by Burcombe and Hollingsworth (1970). The protein content was estimated by following the method of Lowry *et al.*, (1951). The PAGE of proteins was done using 9.3% polyacrylamide gels in disc electrophoretic apparatus according to the method of Davis (1964) and Ornstein (1964). The staining of protein bands was done in Coomassie brilliant blue dissolved in methanol: water:acetic acid (5:5:1 v/v).

RESULTS

A comparison of the total body protein content on fresh weight basis in all nymphal instars as well as in apterae adult of *L. erysimi* showed a negative correlation with age, where the protein content declined from 0.123 mg/mg in the first instar to 0.104 mg/mg in the adult apterae. The treatment with methoprene for 48hrs resulted in an increase in protein content in all nymphal instars and apterae adults, even at the lowest tested concentration (Fig 1).

The PAGE analysis of proteins of the treated nymphal instars and adults revealed that methoprene influenced the number of bands, mobility of bands and the intensity of bands. The total number of bands varied from 10–12 during normal development of mustard aphid, whereas after methoprene treatment, the number varied from 10–16 with the maximum number in the fourth nymphal instar (16 bands) (Fig 2).

DISCUSSION

The total body protein content of *L. erysimi* on the basis of fresh weight decreased as the development proceeded, this might be due to an increase in the water and lipid content of this insect as it is a filter feeder (Rup and Sohal, 1987) 1988.

A significant increase in the protein content with methoprene treatment in all nymphal instars and apterae adults compared to control at each stage clearly indicated that it influenced the protein synthesis of this insect in some way. Increase in the protein content after methoprene treatment have been observed in the cases of *Spodoptera litura, Bombyx mori* and *Lymantria dispar* by Sundaramurthy and Ahmed (1978), Kajiura and Yamashita (1989) and Davis *et al.*, (1990), respectively. Elliot and McDonald (1976) and Scheller and Bodenstein (1981) attributed the increase in the protein content to the modification of protein and RNA synthesis in *Aedes aegypti* and *Calliphora vicina*, induced by Altosid/Methoprene.

On the other hand, Adams *et al.*, (1989) and Kempa–Tomm *et al.*, (1990) have even tried to specify the influence of methoprene on proteins by reporting that methoprene caused the increase in the synthesis of RNA which stimulated the vitellogenin gene, hence an increase in the vitellogenin in *Musca domestica* and *Gryllus bimaculatus*.

The appearance of increased number of protein bands and change in their mobility as well as intensity after methoprene treatment indicated that in addition to stimulating the protein synthesis, it also initiates the synthesis of some new proteins in *L. erysimi*. These findings get corroborated by the results of Mjeni and Morrison (1976), Kajiura and Yamashita (1989) and Bradfield *et al.*, (1990) as they also recorded an increase in the number of protein bands in the male larvae of *Phormia regina, Bombyx mori* and *Blaberus discoidalis*, respectively, with methoprene treatment. Cotton and Anstee (1991) attributed the appearance of new proteins in precocious females of *Locusta migratoria* to the activation of additional chromosomal loci with methoprene treatment. On the other hand, the differential gene activity was attributed by Bassi and Feir (1971) to the change in intensity of the protein bands in *Oncopeltus fasciatus* under the influence of juvenile hormone.

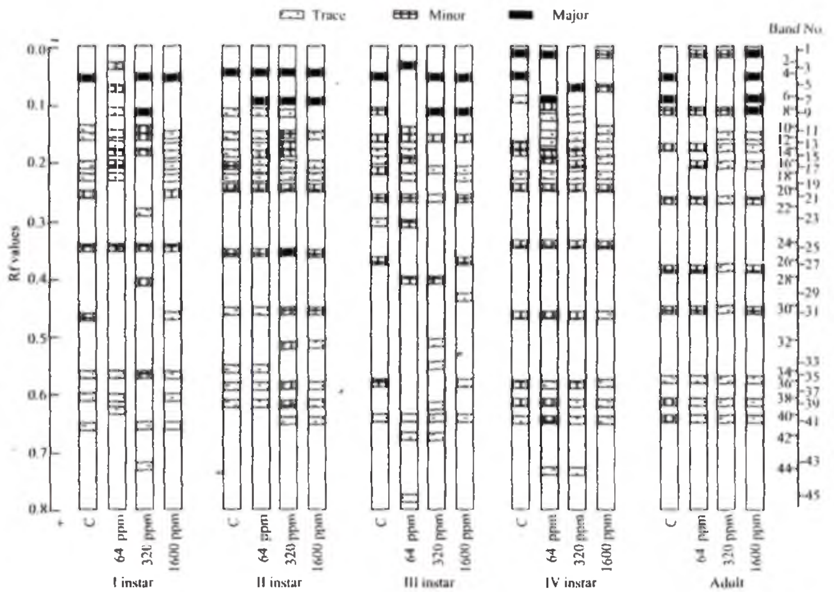

Fig. 2: Electrophoretic banding pattern of proteins of *L. erysimi* after treatment with methoprene.

ACKNOWLEDGEMENTS

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Entomon 23(1): 17–21 (1998)

MAC–positive Peptidergic Neurons in the Red cotton Bug, *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae)

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Abstract: Monoclonal antibodies developed against peptidergic neurons in the neuro endocrine tissues of Colorado potato beetle, *Leptinotarsa decemlineata* referred as MACs were used to immunocytochemically localise similar peptidergic neurons in the brain of *Dysdercus cingulatus*. MACs –3, –13 and –18 are used in the study. Putative peptidergic neurons are localised with MAC –3 fraction. Immunoreactive neurosecretory neurons were revealed in the medial groups of NS cells in the protocerebrum of brain and also in the corpora cardiaca – allata region. It implicates that these immunoreactive substances represent neuropeptides or precursors of different kinds.

Keywords: *Dysdercus cingulatus, Leptinotarsa decemlineata* immunocytochemistry, monoclonal antibodies, neuropeptides.

INTRODUCTION

Insect neuroendocrinology is usually studied either by the localization of peptidergic centres or by determining the physiological function or chemistry of neuropeptides seldom by a combination of disciplines. For elucidation of the true structure of hitherto unidentified 'new' insect neuropeptides, Verhaert *et al.*, (1986) have designed a novel screening method to facilitate the primary detection of neuron–specific antibody secreting mouse–mouse hyrbidoma clones obtained after immunisation with neuronal tissue homogenates. It promised to be very useful to discriminate amongst the wide range of antibodies to various kinds of materials produced by hybridomas by detecting monoclonal antibodies directed against factors contained in well defined tissues in which one is interested. The monoclonal antibody technology is a relatively new development in insect neurobiology (Schooneveld and Smid, 1989a; Schooneveld and Smid, 1989b). It allows one to pick out specific antibodies that react with a single antigenic determinant found in the crude immunizing homogenates.

Schooneveld *et al.*, (1989) prepared a panel of monoclonal antibodies anti–Colorado potato beetle antigens (MACs) for the demonstration of putative neuropeptides in the

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central nervous system and identified peptidergic neurons in *Leptinotarsa decemlineata*. Most of the MACs recognised the content of secretory granules in the corpora cardiaca, presumably neuropeptides. Release seemed evident for the NSCs in the protocerebrum (Schooneveld, 1970; Schooneveld, 1974). His library of monoclonal antibodies included MACs 1–18. Of these 13 of them stained putative peptidergic neurons in different locations of the Colorado potato beetle. Many MACs immunostain different populations of peptidergic neurons of different ganglia of the CNS, especially in the brain. Most of them revealed specific types of peptidergic neurons. Although speculative, randomly generated antigen–specific MABs could be used to unravel hitherto unknown functions of peptides and other antigens in insect tissue (Schooneveld and Smid, 1989a). The neurons are tentatively called peptidergic if a distant immunoreactivity is associated with a cellular product, even though the presence of peptide remains to be demonstrated. The set of MACs obtained is useful for neuro–anatomical studies, for characterising the secretory products and for a further delineation of peptidergic communication channels in the insect body. The present study is an attempt to localise the peptidergic centres in the brain of *D. cingulatus* using MACs.

MATERIALS AND METHODS

Fifth instar 3 day old nymphs of the cotton bug, *Dysdercus cingulatus* Fabr were used for the present study. Insects of appropriate age groups required for the studies were obtained from the stock colony maintained under controlled conditions in our lab for more than the last 20 years. Brains and corpora cardiaca–allata complex were dissected out and fixed either in aqueous Bouin's fluid or in GPA mixture (Boer, *et al.* 1979,) and paraffin sections were made out of these.

MACs are monoclonal antibodies prepared from Colorado potato beetle *Leptinotarsa decemlineata*. MACs –3, –13 and –18 fractions were used for the study. They were a kind gift from Dr. H. Schooneveld, (The Netherlands). Labelled antibody enzyme method is used for immunocytochemical study. The sections after deparaffination were downgraded upto 70% ethanol, washed in phosphate buffered saline (PBS) two times and then treated with selected dilutions of monoclonal antibodies, 30 minutes at room temperature and subsequently in a refrigerator overnight. After 24 hours, washed in PBS, treated with peroxidase conjugated rabbit immunoglobulins to mouse immunoglobulins. Then washed in PBS two times and developed in 10% Diaminobenzidine (DAB) diluted to 0.05% with PBS to final volume and added 200 μl H_2O_2 for five minutes. Washed in tap water and counter-stained in Mayer's haematoxylin and mounted in DPX.

Negative Control for MACs :

The control sections of brain were processed by omitting the first antibody to check unspecific binding of compounds.

RESULTS

In *Dysdercus cingulatus* the NSCs are located in two groups, on either side of median line, on the mid-dorsal side of brain (Fig. 1). They are of A, B and C types. When monoclonal antibodies were used to localise the putative peptidergic centres of the

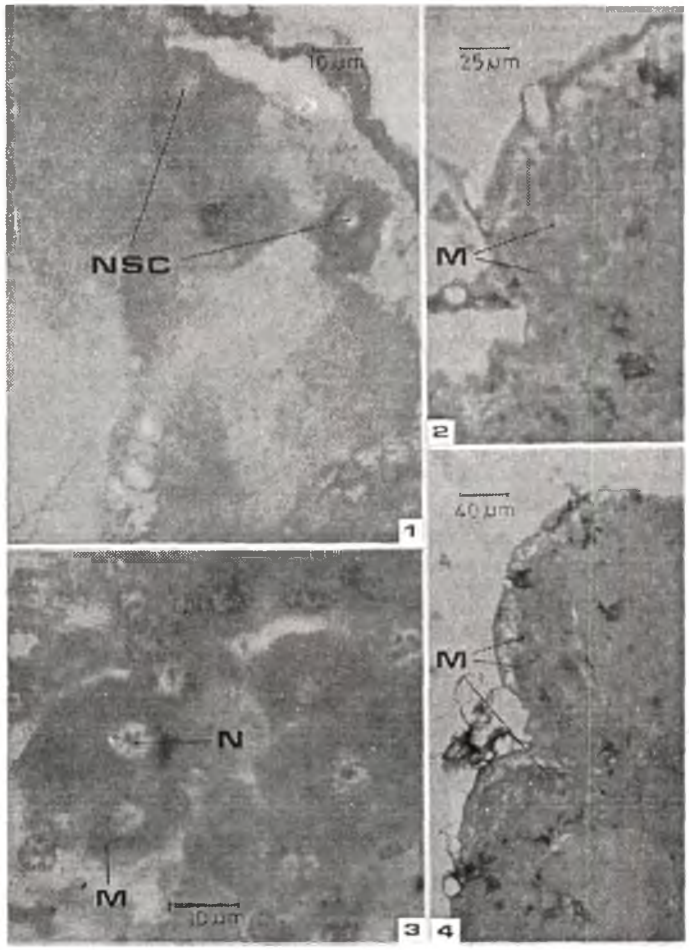

Fig. 1. T. S. of brain of *D. cingulatus* showing MNSCs – PF staining; Fig. 2. T. S. of brain showing MAC –3 positive cells; Fig. 3. Enlarged view of MAC –3 positive cells; Fig. 4. T. S. of brain showing putative peptidergic neurons in the pars intercerebralis region stained with MAC –3.
NSC – Median Neurosecretory Cell; M – MAC–3 Positive Cell; N – Nucleus

brain, the Bouin fixed sections showed no antigenicity. Glutaraldehyde fixed tissues also showed no antigenicity with MACs –13 and –18. But MAC –3 have shown positive results with the brain of *Dysdercus*. 1–7 numbers of MAC –3 positive cells could be observed on either side of the pars intercerebralis region in adjacent histological sections of brain (Fig. 2). They were about 20 μ in diameter and nuclear diameter varied about 5–10 μ and are somewhat oval or round in shape (Fig. 3). Some of the MAC –3 positive cells are very small and their cell diameter is 10–15 μ and nucleus 5 μ (Fig. 4). The cells appeared in brown colour due to DAB staining. The immunoreactive cells correspond to the neurosecretory cells stained by classical neurosecretory stains. Some cells of the CC–CA were also stained by MAC –3. These immunoreactive cells represent neuropeptides or precursors of different kinds.

Negative control slides appeared in blue colour due to haematoxylin counter-staining.

DISCUSSION

Using monoclonal antibodies several neuroendocrine centres within the central nervous system can be discovered using immunocytochemical methods (Schooneveld, 1990). The present report of MAC –3 immunoreactive cells in *D. cingulatus* brain is interesting in that antibodies of the brain homogenate of one insect species cross-reacted with the neurons of another insect species belonging to another order *i.e.*, MACs from a beetle gave positive results with neurons from a bug. So the MACs may not be species specific. MAC –3 stained homologous peptidergic neurons in *D. cingulatus*. The immunoreactive substances represent neuropeptides or its precursors.

In the present study in *Dysdercus* brain with MACs (MAC –3, –13 and –18) aqueous Bouin's fluid and GPA mixture were used as fixatives. Only when GPA fixation is used, the immunoreactive cells to MAC –3 is revealed in the brain. This showed a specificity for fixation as well. Veenstra *et al.* (1984) also suggested that a positive reaction of a particular group of immunoreactive cells depend strongly on both the fixation procedures and the antiserum used.

As peptidergic messengers and representatives of diverse animal phyla often make use of similar type of neuropeptides, MABs from one invertebrate species could well be of use to unrelated vertebrates also (Schooneveld and Smid, 1989a; Schooneveld and Smid, 1989b). They also found that MAB from pond snail *Lymnaea stagnalis* (mollusca) called ALMA –3 immunostained certain populations of peptidergic neurons in the Colorado potato beetle. Some of the MABs developed by Schooneveld *et al.* (1989) immunostained specific neuron populations in the migratory locust and in pond snail. Likewise MABs against molluscan cardioactive peptide proved to reveal neurons in several classes of invertebrates including insects (Masinovsky, 1988). MABs against Drosophila CNS revealed compounds in the brain of humans, indicating a broad distribution of antigens among representatives of different animal phyla (Miller and Benzer, 1983). Muraleedharan *et al.* (1994) demonstrated that the midgut of the Colorado potato beetle contain endocrine cells that can be immunostained with monoclonal antibodies namely MACs –3, –8, –9 and –18. Therefore MABs may well be of use for more general neuroanatomical purpose in and outside the invertebrate groups especially for the identification of peptidergic neurons.

Following the technique of Schooneveld *et al.* (1989), other investigators also developed hybridomas secreting neuron–specific antibodies. Denburg *et al.* (1986) working with CNS of *Periplaneta americana*, Fujita *et al.* (1982) with *D. melanogaster*, Hishinuma *et al.* (1988) with *Manduca sexta* all illustrating the general usefulness of this technique. The antigenicity of tissues and cells differs markedly among species using MACs. Raikhel *et al.* (1986) have produced a library of monoclonal antibodies against yolk proteins of mosquito *Aedes aegypti*.

Monoclonal antibodies can serve as powerful tools with which one could study the structural and functional properties of yolk proteins, as well as to develop highly sensitive quantitative assays with defined and reproducible characteristics. The antigenicity with MACs in *D. cingulatus* no doubt gives the regionalised distribution of many neuropeptides which provides clue to peptide function.

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Entomon 23(1): 23–28 (1998)

Structure and Formation of Spermatophore in A Tick, *Haemaphysalis intermedia* (Acarina :Ixodidae)

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Abstract: the spermatophore formation and the transfer mechanisms together with the mating behaviour of a tick, *Haemaphysalis intermedia* have been studied. The wall of the spermatophore consists of two envelopes one within the other. The internal envelope encloses a large space occupied by sperm bundles which are invariably three in number. The highly dilated and transparent distal portion of the vasa deferentia is concerned with the formation of spermatophore wall material and hence referred to as spermatophorogenetic organ. Histological studies show that the entire spermatophorogenetic organ consists of large glandular cells. The cells of the wall in this portion give rise to membranes of mucopolysaccharide nature by delamination from the free margins. At the junction between the distal and middle portions of the spermatophorogenetic organ there is a mechanism for the injection of sperm bundles into the prospective inner envelop material.

Keywords: *Haemaphysalis intermedia*, spermatophore, spermatophorogenetic organ.

INTRODUCTION

It is well known that ticks employ spermatophores in sperm transfer (Feldman-Musham and Borut 1978; Oliver, 1982; Singaravelu and Mahalingam, 1996). Previous workers have recorded two types of spermatophore formation among ticks. Nuttall and Merriman (1911), Wagner–Jevseenko (1958) and Tatchell (1962) have recorded that the spermatophore in soft ticks is formed inside the male body and extruded as fully formed unit, but Feldman–Muhsam and Borut (1983) and Oliver (1988) observe that the spermatophores are formed outside the male body. On the basis of such evidences the author considers that the spermatophore are formed inside the male body.

MATERIALS AND METHODS

The materials employed in the present investigation were ticks of the species *Haemaphysalis intermedia* collected from the cattle, sheeps and goats reared at Amirthi forest in the vicinity of Vellore research station. A culture of these ticks was maintained in the

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Fig. 1. Photograph of a spermatophore of *Haemaphysalis intermedia* (x 50).
Fig. 2. Obilgue section of a spermatophore of *Haemaphysalis intermedia*, just emerged out of the male genital opening, (x 60).

laboratory (Ilkal and Dhanda, 1981). The two kinds of males, *i.e.*, the early adults, (the final instar males before engorgement) and the late adults, (those after engorgement) (Dumser and Oliver, 1981) were dissected out in arachnid saline (Elshoura, 1988). Figures of the male reproductive system were drawn with the help of a camera lucida. Sections were cut from specimens preserved in 5% neutral formalin or Bouin's fluid. Sections were prepared by the conventional paraffin embedding in celloidin and wax. For histological studies the sections were stained in Mallory's triple stain.

RESULTS

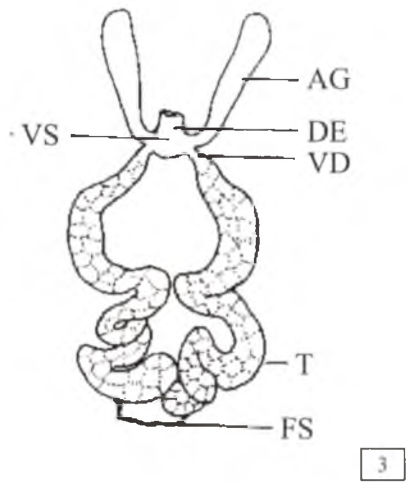
The structure of the spermatophore:

In *H. intermedia* a fully formed spermatophore is an oval reniform structure and it is yellowish brown in colour (Fig. 1) The size of spermatophore varies from $1000 \pm 25\mu m$ lengthwise; about $850 \pm 30\mu m$ in breadth-wise. Sections passing across a spermatophore reveal that the wall of the structure is made up of two envelopes, one within the other (Fig. 2).

The external envelope is wavy; the waves are smooth in most places and deep in some portions and the wall of external envelope is homogeneous and it measures about $150\mu m$ in thickness, while the internal envelope is oblong in shape and its wall is mostly parallel to external; never they are confluent with each other. The space in between the external envelope and internal envelope is narrow. The wall of the internal envelope is thicker than that of the outer one and it measures about $250\mu m$. The internal envelope encloses a large space occupied by sperm bundle which are always three in number. The sperm bundles are unequal in size and irregular in shape with each of them, bounded externally by a thin but distinct membrane. Each bundle encloses spermatids which are more concentrated in some zones and sparce in other places (Fig. 2).

Spermatophore formation:

The male reproductive organs of *H. intermedia* in the early adult stage consist of a pair of testes. Each testis is a long convoluted and filamentous structure connected



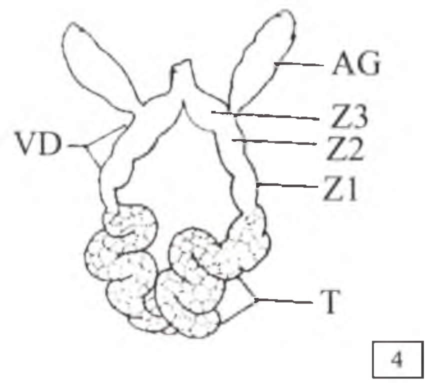


Fig. 3. Diagrammatic illustration of the reproductive system of an early adult male of *Haemaphysalis intermedia*. AG : Accessory gland; DE : Ejaculatory duct: FS : Filamentous strand: T : Testis; D : Vasa deferentia; VS : Vas deferens.

Fig. 4. Diagrammatic illustration of the reproductive system of a late adult of a late male of *Haemaphysalis intermedia*. AG : Accessory gland; T : Testis; VD : Vasa deferentia; Z1 : Zone – 1; Z2 : Zone – 2; Z3 : Zone – 3;

on its anterior extremity to the vasa deferentia. The vasa deferentia of both sides unite, giving rise to a common vas deferens which is in communication with the ductus ejaculatorius. The ejaculatory duct opens into the external genital orifice (Fig. 3).

In the late adult stage the vasa deferentia get more enlargement compared to all other organs (Fig. 4). The lumen of the expanded distal portion of the vasa deferentia becomes very broad at the junction where it meets the testes and it becomes highly coiled, the distal portion of the vasa deferentia is highly dilated and transparent. This is the region in which the spermatophores are formed, hence it is referred to be "spermatophorogenetic organ" in the sequel. There is a pair of accessory glands in association with the middle portion of the vasa deferentia.

Histological studies show that the entire spermatophorogenetic organ consists of large glandular cells. The cells of the wall in this region give rise to the membranes of mucopolysaccharides nature by delamination from the free margins (Fig. 5). Sperm masses swathed by the membranes formed in the anterior portion of the vasa deferentia and the sperm bundles are moved forward to the middle portion of the vasa deferentia. Accessory glands are attached in this region one on each side and these glands give rise to membranes which form the internal envelope enclosing the sperm bundles.

The junction between the distal and middle portions of the spermatophorogenetic organ is narrow; here after called 'neck' demarcating the two segments which are functionally different (Fig. 6). Within the neck is found a valve – like organ which serves to introduce the sperm bundles into the prospective inner envelope material. The cells of the distal portion of the vasa deferentia form membranes which swathes loosely

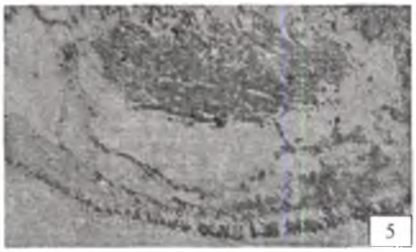


Fig. 5. T. S. through the Zone – I of the vasa defeientia of a late adult male of *Haemaphysalis intermedia.*, showing the process of swathing of the delaminating membranes, from the well, around a sperm bundle while forming the bounding membrane. stained in Heidenhain's haematoxylin. (x 120).
Fig. 6. L. S. through the valve of the vasa deferentia of a late adult male of *Haemaphysalis intermedia* stained in Heidenhain's haematoxylin. (x 120).

around the internal envelope and give rise to external envelope; the spermatophore now has all the components.

Histochemical reactions and staining properties disclose that the membranes formed by the accessory glands are released into the middle portion of the vasa deferentia and membranes formed by the distal portion of vasa deferentia showed positive reactions to tests for proteins as well as to those for aromatic groups. These membranes were non–reactive to tests for quinones and also to the techniques for lipid. They are, however, distinctly positive to all the reactions for acid mucopolysaccharides.

DISCUSSION

The tick spermatophore, when it emerges out of the male genital opening, has an outer bulb and a bilobed membranous structure in the neck enclosing the former like a stopper. The rims of these two being tightly sealed together so as to form a single structure; the spermatids occupy the entire space between the outer bulb and the inner bulb (Tatchell, 1962; Feldman-Musham and Borut 1978). Feldman–Muhsam (1967) has proposed the terms ecto and endo spermatophores for their bilobed structure. Many structures such as vase, adjacent body, sponge, tubule and arrow have been found in 8 ixodid species but none of these organelles were found in any species of Argasidae (Feldman-Mushsam and Borut, 1984).

The foregoing facts indicate that the structure of the spermatophore may be varying among the different species of ticks, as in the case of insects (Jaiswal and Naidu, 1976; Yang and Chew 1978; Schaller, 1980). Oliver *et al.*, (1974) further found a thin membranous sac within the ectospermatophore; the spermatids and other materials are present in this sac.

The spermatophore of *H. intermedia*, in the present investigation, resembles in many respects that of the hard ticks studied by Oliver (1982). The two envelopes of the spermatophore one within the other correspond in their positions to the ecto and endospermatophere membranes of the 2 species of hard ticks studied by Oliver (1982).

Another variation pertains to the mass of spermatids. The spermatids in soft ticks like *Argas persicus* fill up evenly the whole of the space inside the spermatophore; the sperm mass is bounded by a tenuous membrane (Tatchell, 1962). But in the case of

a hard tick *Dermacenter occidentalis* the spermatids are confined to the base of the membranous sac of ectospermatophore, no bounding membrane is found (Oliver, *et al.*1974,).

In the present study, the spermatids are found as bundles inside the spermatophore and each of them bundled by a membrane of its own. There is a controversy about the site of spermatophores formation in the acarines. The earlier workers, Nuttall and Merriman (1911), Robinson (1942), Wagner – Jevseenko (1958) and Tatchell (1962) have recorded that the spermatophore in soft ticks is formed inside the male body and extruded as a fully formed unit. But later workers, Feldman – Musham and Borut (1983), Oliver (1988) observe that the spermatophore is formed outside the male body.

In the present investigation, the recorded results reveal that the spermatophores are formed within the male genital tract. Each and every part of the male reproductive system in this ixodid tick contributes to the formation of spermatophore. There is a valve like structure inside the vasa deferentia; there are probabilities, by comparison with other groups of arthropods (Sundararajulu, 1970), that the valve may play a role in regulating the passage of sperm bundles while the prospective internal envelope components encircle them.

The internal and external envelopes are formed inside the vasa deferentia, the cells of whose wall give rise to the requisite material by the delamination as in the case of formation of the peritrophic membrane in many species of insects (See (Imms, 1957)). The histological properties as well as the histochemical reactions of the accessory glands and the vasa deferentia are in conformity with that of the walls of the spermatophores in the case of the tick, *H. intermedia*. (Singaravelu, 1991).

These facts denote that the structural features and formation of the spermatophore vary among the different groups of ticks.

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Use of Freezer Stored and Coddled Pupae of *Exorista bombycis* (Diptera : Tachinidae) for Culturing its Parasitoids

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Abstract: Hymenopteran ectopupal parasitoids of *Exorista bombycis* Louis namely, *Nesolynx thymus*, *Pachycrepoideus veerannai* and *Dirhinus anthracia*, oviposited and developed successfully on freezer stored and coddled host pupae. *N. thymus* and *P veerannai* could be cultured on pupae stored for 64 days. However, a reduction in the total progeny was observed when *P. verrannai* was cultured on pupae freezer stored for 64 days and beyond. By coddling the host pupae before culturing the host pupal development gets stalled and adult host emergence which is undesirable while culturing, is prevented.

Keywords: Coddling, *Dirhinus anthracia*, Freezer Storage, *Nesolynx thymus*, *Pachycrepoideus veerannai*.

INTRODUCTION

Indian uzi fly *Exorista bombycis* Louis (Diptera : Tachinidae) is an endoparasite of mulberry silkworm *Bombyx mori* L. (Lepidoptera : Bombycidae). It is a serious pest of mulberry silkworm inflicting a sizable damage to the sericulture industry in India (Kumar Pradeep and Jolly, 1986). In recent years a substantial effort towards achieving biological control of uzi fly has resulted in recording and conducting studies on its parasitoids. Some of the parasitoids so recorded are also tested in the field as a component in integrated management of uzi fly (Jyothi, 1994). However, mass rearing of its parasitoids is limited by the availability of large number of host pupae at a critical time they are needed. This problem could be overcome by way of using freezer stored host material.

Freezer stored eggs of several species have been successfully used to rear egg parasitoids (Drooz and Weems, 1982: Powel and Shepard, 1982). Rearing of pteromalid parasitoids *Pachycrepoideus vindimiae* (Rondani), and *Muscidifurax zaraptor* Kogan and Legner, on freeze killed house fly pupae is reported (Pickens and Miller 1978; Peterson and Mathews, 1984). There are also reports of utilising coddled host material to

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increase mass culturing efficiency of *Dahlbominus fuscipennis* a parasitoid of *Diprion* and *Neodeprion* (Coppel and Mertins, 1977).

The ability to store normal/coddled host pupae in freezer for long periods of time and their subsequent use as hosts would be of great benefit when mass production of the parasitoids is required.

In the present investigation attempts are made to determine the ideal duration of storage of host pupae for effective utilisation in multiplication of three hymenopteran ectopupal parasitoids of uzi fly namely, *Nesolynx thymus* (Girault) (Eulophidae), *Dirhinus anthracia* Walker (Chalcididae) and *Pachycrepoideus veerannai* (Narendran and Anil) (Pteromalidae).

MATERIALS AND METHODS

To determine whether parasitoids were able to successfully develop on the freezer stored *E. bombycis* pupae, the pupae were stored in the freezer for different durations such as 1, 2, 4, 8, 16, 32, 48, 64 days at 0° C. These pupae were thawed for 4–6 hours after removal from the freezer and exposed to parasitoids for infestation.

Two pairs each of freshly emerged *D. anthracia* and *P. veerannai* and five pairs of *N. thymus* were allowed to oviposit separately on freezer stored pupae in batches of 100 each in three replications, for 24 hours. After 24 hours the parasitoid adults were removed. Infested host pupae were observed for host and parasitoid adult emergence. The parasitoid adults so emerged were counted and sexed. Developmental time of the parasitoids and percentage of parasitism of each female parasitoid per day were compared between normal and freezer stored hosts. Data were analysed statistically by ANOVA.

Similarly, one day old host pupae were 'coddled' *i.e.*, kept immersed in warm water at 40° C, 45° C and 50° C for five minutes (Coppel and Mertins, 1977). The pupae were air dried and subsequently used as host material for culturing parasitoids using the procedure similar to the one used for freezer stored hosts pupae.

Efficiency of the adult parasitoids obtained from freezer stored pupae was tested as follows. Five pairs of the parasitoid adults of all the three species under investigation reared on freezer stored hosts of different duration of storage were exposed to 100, 3–4 day old uzi fly pupae for 24 hours. The parasitoid adults that emerge from these parasitised hosts were sexed and counted. Percentage of parasitism was calculated by comparing the number of hosts that were infested, to those that were exposed.

Similarly, the progeny obtained from coddled pupae were tested for their ability to reproduce.

RESULTS

Parasitoids *N. thymus*, *D. anthracia* and *P. veerannai* were able to parasitise and successfully develop on the host uzi fly pupae of different durations of freezer storage producing fertile offspring.

Nesolynx thymus

Observations made on culturing *N. thymus*, on freezer stored pupae are given in Table 1. Number of male progeny recovered from host pupae stored for different durations

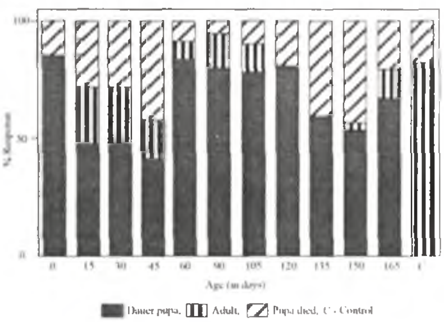

Fig. 1.

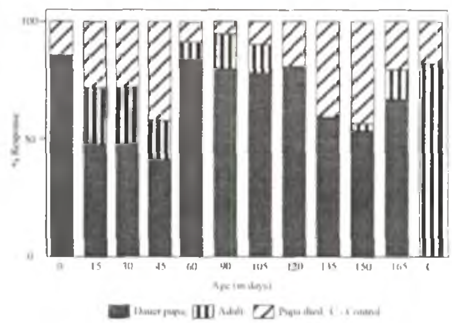

Fig. 2.

was significantly smaller than those obtained from normal hosts. Highest number of females though not statistically different from control was obtained from pupae stored for 2 days (82.15 ± 12.02). Percentage of parasitism was higher on freezer stored pupae compared to normal hosts. However, the difference was not statistically significant. Time taken for development was higher on stored hosts. Progeny of female oriented sex ratio was obtained on both normal and stored pupae.

Table 1. Effect of cold storage of *Exorista bombycis* pupae at 0° C on culturability and development of *Nesolynx thymus*

Days of Storage	No. of male Progeny	No. of female Progeny	% Parasitisation /female/day	Developmental Time (Days)		Sex Ratio (M : F)
				Males	Females	
1	1.35±0.33	60.40±8.38	0.55±0.19	15.80±0.75	15.80±0.75	1 : 44.74
2	1.65±0.30	82.15±12.02	0.55±0.10	15.90±0.60	15.90±0.64	1 : 49.78
4	1.75±0.44	66.50±15.44	0.80±0.63	16.40±0.69	16.40±0.69	1 : 38.00
8	1.74±0.68	73.85±11.69	0.55±0.41	17.40±0.55	17.40±0.55	1 : 42.44
16	1.80±0.48	58.85±5.32	0.60±0.16	18.50±0.84	18.50±0.84	1 : 32.69
32	2.80±1.34	62.65±3.19	0.55±0.10	18.40±0.77	18.40±0.77	1 : 22.37
48	1.50±0.36	57.70±3.66	0.65±0.25	19.10±0.87	19.10±0.87	1 : 38.46
64	2.20±0.23	62.05±5.17	0.60±0.16	18.80±0.63	18.80±0.63	1 : 28.20
normal	4.00±1.26	72.40±6.03	0.54±0.00	16.60±1.04	16.60±1.04	1 : 18.10
LSD(5%)	0.92	11.03	NS			

Note : Normal means pupae maintained at room temperature
NS means not significant

Observations made on the fecundity (progeny obtained /female/day), percentage of parasitism and sex ratio of the progeny of adult females cultured on freezer stored hosts are presented in Table 2. Fecundity (number of progeny), percentage of infestation and sex ratio of adult parasitoids obtained from stored host pupae were comparable to flies cultured normally.

Table 2. Total progeny, percentage parasitism and sex ratio of the progeny of Nesolynx thymus obtained from freezer stored hosts

Days of storage	Total progeny /female/day	% Parasitism	Sex ratio (M : F)
1	158.35±13.43	0.55±0.10	1 : 23.40
2	165.45±16.34	0.60±0.16	1 : 14.34
4	125.23±15.34	0.40±0.25	1 : 15.36
8	171.00±14.34	0.55±0.18	1 : 12.35
16	204.00±20.13	0.45±0.19	1 : 23.45
32	235.45±23.13	0.55±0.10	1 : 18.56
48	184.23±22.45	0.45±0.41	1 : 20.19
64	234.25±28.16	0.60±0.18	1 : 21.34
Normal hosts	213.65±18.13	0.54±0.45	1 : 18.13
CD (5%)	NS	NS	

Note: NS means satistically not significant

Pachycrepoideus veerannai

Emergence of male progeny was significantly higher on normal host pupae compared to freezer stored hosts. Emergence of adult females was higher on normal host pupae but did not vary significantly from number of female progeny obtained on the hosts stored for various durations. Developmental time for both male and female parasitoids was longer on freezer stored hosts. Sex ration of the progeny was female oriented on both normal and stored pupae (Table 3).

Table 3. Effect of cold storage of *Exorista bombycis* pupae at 0°C on culturability and development of *Pachycrepoideus veerannai*

Days of Storage	No. of male Progeny	No. of female Progeny	% Parasitisation /female/day	Developmental Time (Days)		Sex Ratio (M : F)
				Males	Females	
1	1.15±0.25	2.12±0.25	3.50±0.40	22.50±0.22	24.70±0.48	1 : 1.84
2	1.62±0.62	3.12±0.62	4.75±0.16	22.60±0.69	24.90±0.56	1 : 1.92
4	2.21±0.85	3.37±1.02	5.50±1.00	23.00±0.94	25.70±0.67	1 : 1.58
8	1.50±0.40	3.37±0.85	4.87±1.25	23.80±0.63	25.60±0.51	1 : 2.24
16	1.87±0.85	2.50±0.95	3.75±1.32	23.30±0.48	26.40±0.69	1 : 1.33
32	1.37±0.75	2.12±1.16	3.50±1.47	23.70±0.94	26.40±0.55	1 : 1.55
48	1.00±0.85	2.60±0.50	3.00±0.81	23.50±0.52	26.40±0.81	1 : 2.60
64	1.12±0.75	1.87±1.04	3.00±1.47	23.20±0.63	26.50±0.52	1 : 1.66
normal	2.37±1.09	3.50±1.22	5.87±2.50	21.50±0.70	24.80±0.63	1 : 1.47
LSD(5%)	0.024	0.75	0.95			

Note : Normal means pupae maintained at room temperature; NS means not significant

Table 4. Total progeny, percentage parasitism and sex ratio of the progeny of Pachycrepoideus veerannai obtained from freezer stored hosts

Days of storage	Total progeny /female/day	% Parasitism	Sex ratio (M : F)
1	4.67±3.20	4.67±3.20	1 : 1.83
2	3.50±1.53	3.50±1.53	1 : 1.24
4	4.63±1.63	4.63±1.63	1 : 2.50
8	4.75±3.45	4.75±3.45	1 : 2.60
16	3.75±2.13	3.75±2.13	1 : 1.40
32	3.50±2.60	3.50±2.60	1 : 2.60
48	4.55±2.50	4.55±2.50	1 : 1.17
64	5.10±3.10	5.10±3.10	1 : 1.45
Normal hosts	5.60±2.15	5.60±2.15	1 : 1.28
CD (5%)	NS	NS	

Note : NS means satistically not significant

Observations made on the fecundity (progeny obtained /female/day), percentage of parasitism and sex ratio of the progeny of adult females cultured on freezer stored hosts are presented in Table 4. The biological parameters of the parasitoids obtained from freezer stored hosts are comparable to those cultured on normal hosts.

Table 5. Effect of cold storage of *Exorista bombycis* pupae at 0° C on culturability and development of *Dirhinus anthracia*

Days of Storage	No. of male Progeny	No. of female Progeny	% Parasitisation /female/day	Developmental Time (Days)		Sex Ratio (M : F)
				Males	Females	
1	4.87±1.93	7.50±1.08	12.37±2.92	17.10±0.08	221.20±1.23	1 : 1.54
2	4.75±2.40	7.00±3.18	11.50±4.18	18.00±0.66	21.20±0.78	1 : 1.47
4	6.37±1.97	8.25±1.70	15.00±2.90	17.30±1.15	20.80±0.62	1 : 1.29
8	6.25±1.93	8.12±0.80	14.12±2.90	18.10±1.50	22.00±0.94	1 : 1.29
16	6.37±0.62	9.12±0.95	16.00±1.41	19.10±0.92	22.90±2.29	1 : 1.43
32	6.62±2.17	8.12±0.35	14.75±2.32	18.50±1.63	22.00±1.33	1 : 1.22
48	6.12±0.63	7.00±1.15	13.37±0.75	19.60±0.97	223.20±1.22	1 : 1.14
64	6.62±1.75	3.12±1.02	9.75±2.32	21.70±0.67	23.10±0.99	1 : 0.47
normal	5.00±1.02	7.75±1.65	12.75±2.53	17.30±0.67	21.20±0.87	1 : 1.55
LSD(5%)	NS	2.15	NS			

Note : Normal means pupae maintained at room temperature
NS means not significant

Dirhinus anthracia

Percentage of parasitism of *D. anthracia* and number of males produced on normal and freezer stored hosts did not vary significantly. However, emergence of female progeny

varied significantly on hosts stored for different durations with highest being 9.12 ± 0.95 /female/day on pupae stored for 16 days.

Total number of progeny/day/female, percentage of parasitism and sex ratio of the adult *D. anthracia* obtained from stored host pupae are comparable with those cultured normally (Table 6).

Table 6. Total progeny, percentage parasitism and sex ratio of the progeny of Dirhinus anthracia obtained from freezer stored hosts

Days of storage	Total progeny /female/day	% Parasitism	Sex ratio (M : F)
1	12.67±3.48	12.67±3.48	1 : 1.75
2	14.38±2.90	14.38±2.90	1 : 1.86
4	15.65±2.98	15.65±2.98	1 : 1.48
8	14.38±0.75	14.38±3.98	1 : 1.75
16	13.47±3.98	13.47±3.98	1 : 1.64
32	12.75±3.48	12.75±4.88	1 : 1.69
48	16.44±4.88	16.44±4.88	1 : 1.54
64	12.45±2.34	12.45±2.34	1 : 1.82
Normal hosts	14.85±2.67	14.85±2.67	1 : 1.75
CD (5%)	NS	NS	

Note : NS means satistically not significant

Host pupal coddling

Coddled host *E. bombycis* could successfully support the growth and development of the parasitoids under investigation. *N. thymus* produced higher number of progeny when cultured on host pupae coddled at 40° C. Sex ratio was female oriented in the progeny produced on both normal and pupae treated at 40° C, 45° C and 50° C. However, proportion of females was highest on pupae coddled at 40° C. Percentage of parasitisation of *N. thymus* on treated and normal hosts did not vary significantly (Table 7).

Table 7. Effect of host pupal codling on the parasitism by *Nesolynx thymus* (Girault)

Treatment	Emergence of F1 Adults		% Parasitism /female/day	Sex Ratio M : F
	males	females		
40° C	15.33±2.51	183.06±39.80	6.66±1.00	1:11.94
45° C	21.66±17.30	124.20±75.33	4.93±1.10	1:5.73
50° C	32.60±10.66	149.93±20.88	4.60±1.94	1:4.59
Control	24.26±15.25	159.25±16.84	3.20±0.63	1:6.56
LSD (5%)	NS	NS	NS	

Note : NS = Not Significant

Host pupal coddling did not affect significantly the emergence of male and female progeny, percentage of parasitism and sex ratio of *P. veerannai* (Table 8).

Table 8. Effect of host pupal codling on the parasitism by *Pachycrepoideus veerannai*

Treatment	Emergence of F1 Adults		% Parasitism /female/day	Sex Ratio M : F
	males	females		
40° C	0.66±0.28	2.00±0.50	2.66±0.28	1:3.03
45° C	0.83±0.28	2.16±0.76	3.00±0.86	1:2.60
50° C	0.83±0.28	1.83±1.04	2.50±0.86	1:2.20
Control	1.75±0.77	2.00±0.50	3.16±0.57	1:1.14
LSD (5%)	NS	NS	NS	

Note : NS = Not Significant

Though host pupal coddling did not affect the percentage of parasitism and emergence of male progeny significantly, sex ratio was reversed when *D. anthracia* was cultured on hosts coddled at 50° C. (Table 9).

Table 9. Effect of host pupal codling on the parasitism by *Dirhinus anthracia*

Treatment	Emergence of F1 Adults		% Parasitism /female/day	Sex Ratio M : F
	males	females		
40° C	2.00±0.50	3.50±0.50	5.50±0.50	1:1.75
45° C	2.50±0.50	3.50±0.50	6.00±0.50	1:1.40
50° C	3.33±0.76	1.33±0.28	4.66±0.57	1:0.40
Control	2.66±0.73	3.83±0.28	6.50±0.86	1:1.43
LSD (5%)	NS	NS	NS	

Note : NS = Not Significant

Adult parasitoids obtained on coddled hosts exhibited normal reproductive behaviour.

DISCUSSION

Results of studies on use of freezer stored pupae *E. bombycis* illustrate that live hosts are not indispensable for the successful development and rearing of the parasitoids, *N. thymus*, *P. veerannai* and *D. anthracia*. The host pupae stored at 0° C for 64 days were suitable hosts for development of all the three parasitoids of uzi fly under investigation. Percentage of parasitism and developmental time required for male and female parasitoids were comparable with a developmental time on live host pupae. The study lasted for approximately 70 days as a result, females used were from different generations which might have been responsible for wide range of sex ratios encountered (M : F was 1 : 18.19 to 1 : 49.78 in *N. thymus*, 1 : 1.33 to 1 : 2.6 in *P. veerannai*

and 1 : 1.36 to 1 : 1.78 in *D. anthracia*). However, the ability to store host pupae in the freezer for long periods of time and subsequent use as host material for culturing, should be of great benefit to biological control programmes using these parasitoids, especially when mass production is required. Chalcid *Brachymeria ovata* successfully developed on freezer stored *Anticarsia gemmatalis* pupae stored upto 120 days. Pteromalids, *Pachycrepoideus vindimiae* and *Muscidifurax zaraptor* developed successfully on freeze killed house fly pupae (Peterson and Mathews, 1984; Pickens and Miller 1978). Thus freezer storage may be a feasible method of accumulating large numbers of hosts over a period of time which, subsequently, could be used for rearing the parasitoids for large scale releases.

Coddled host pupae have been used to rear *Dahlbominus fuscipennis*, parasitoid of *Diprion* and *Neodeprion* to increase their mass rearing efficiency. Percentage of parasitism of *N. thymus* was higher on coddled hos pupae compared to normal hosts. Coddling of the host pupae did not affect the development of *P. veerannai* and *D. anthracia* but for the emergence of only male progeny on pupae treated at 50° C. Since larval proteins of *E. bombycis* get denatured due to coddling, emergence of the host adult is prevented. Thus coddling of the host pupae could be utilised for avoiding host adult emergence. This will prevent the escape of adult flies while handling the culture and also save labour in removing the hosts from rearing containers since the adult hosts left in the rearing containers will contaminate the same with the excrements and removal of the hosts cadavers from the rearing containers is a messy affair.

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On Five New Species of *Tetramorium* (Hymenoptera: Formicidae: Myrmicinae) From India

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Abstract: Five new species of *Tetramorium* viz. *T. cordatus*, *T. keralensis*, *T. malabarensis*, *T. petiolatus* and *T. sentosus* are described and illustrated. The affinities of these species with closely related ones are also discussed.

Keywords: Five New Species, *Tetramorium*, India.

While studying the Formicid fauna of Malabar (Sheela & Narendran, 1997), we came across five interesting species of Tetramorine ants which do not fit to the key to species of *Tetramorium* by Bolton (1976, 1977), Bingham (1903) and to any description of any known species of *Tetramorium* including that of Mathew (1980).

1. *Tetramorium cordatus* sp. nov. (Figs. 1-3)

Holotype Worker: *Head* finely reticulate, posteriorly transverse in front view; sides straight, mandibles feebly striate with three acute apical teeth followed by three or four small denticles; anterior margin of clypeus nearly arched, with a thin flange, clypeus broad, antero–medially a little depressed; a strong median carina and two or three pairs of feeble lateral carinae present on mid lobe, frontal area not distinctly defined; posterior clypeal margin not distinct; frontal lobes short, wide apart, frontal grooves reaching beyond eye by 1.2x length of eye, a median longitudinal carina separates scrobe into two distinct grooves for reception of scape and flagellum separately; scrobe deep rugulose, posterior margin clearly defined. Antennae 12–jointed, scape slender, flagellar segments 2-7 transverse, club formed of apical 3 joints, which is longer than remaining flagellar segments together (13:8); terminal club segment : preceding two together = 7:5. Eyes prominent, oval lateral, placed at mid transverse line, in profile more to anterior portion.

Thorax: Finely reticulate, laterally a little compressed, anterolateral corners of pronotum rounded; no sutures on thorax, in profile evenly convex; propodeal spines in dorsal view a little longer than distance between its bases (4 : 3.5); in profile its length : basal width = 4 : 2; metapleural lobes triangular, flat, acute at apex, but distinctly shorter

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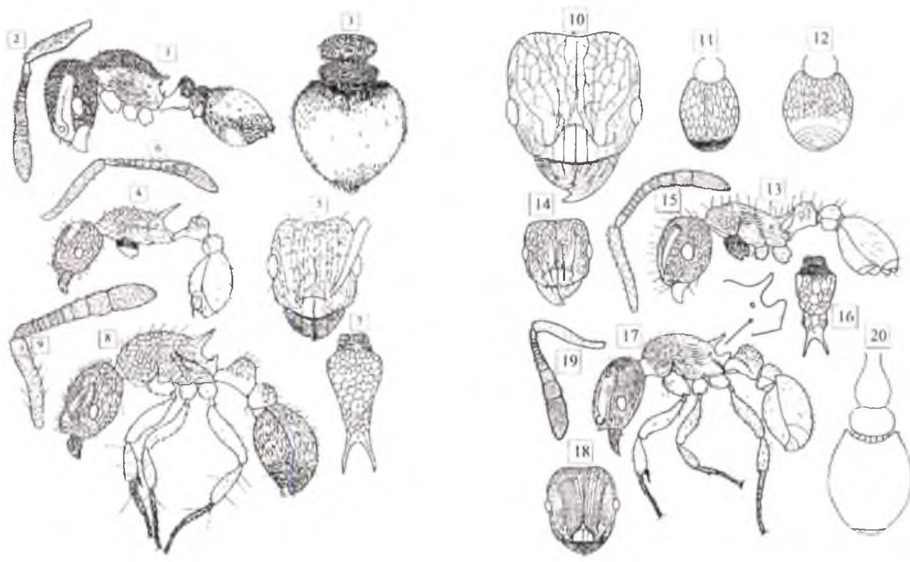

Figs. 1-3- *Tetramorium cordatus* sp. nov. 1–body profile, 2–antenna, 3–abdomen dorsal view; Figs. 4-7- *Tetramorium keralensis* sp. nov. 4– body profile, 5–head front view, 6–antenna, 7–thorax dorsal view; Figs. 8-12-*Tetramorium malabarensis* sp. nov. 8–body profile, 9–antenna, 10–head front view, 11–gaster dorsal view, 12–gaster ventral view; Figs. 13–16- *Tetramorium petiolatus* sp. nov. 13–body profile, 14–head front view, 15–antenna, 16–thorax dorsal view; Figs. 17-20- *Tetramorium sentosus* sp. nov. 17–body profile, 18–head front view, 19–antenna, 20–abdomen dorsal view.

than propodeal spines. Legs smooth, femora and tibiae swollen, tibial spur on mid and hind legs not conspicuous.

Abdomen: Pedicel reticulate, ventrolateral portions smooth, petiole more stoutly built with a short peduncle in front; petiole node: peduncle 5 : 3; petiole higher in a position than postpetiole; dorsal and anterior margins meet smoothly so that no angle at their junction, posteriorly no constriction on petiole, anterior margin almost vertical, dorsally convex (Fig. 3); in dorsal view post petiole broader than petiole (9.5 : 9), and both nodes anteroposteriorly compressed, transverse; petiole length : breadth 4 : 9; postpetiole 3.5 : 9.5. Gaster cordate, anterior 1/3 of T1 finely longitudinally striate, remaining portions smooth; junction between gaster and postpetiole not conspicuous due to presence of a thick felt of pubscence; T1 covering almost entire dorsum, in profile gaster slightly convex above and highly convex below.

Head, thorax and pedicel yellowish brown; gaster brownish black; antennae, mandibles and legs pale yellow; whole body except flagellum of antennae and tarsi of legs with abundant, short, trifid and quadrifid hairs; flagellum and tarsi with minute single pubescence.

Measurements: Total length 2.2 mm; Head length– 0.59mm; Head width - 0.59mm; Cephalic index–100; eye diameter– 0.12mm; Scape length–0.34mm; Scape index–58; Pronotal width–0.46mm; Alitrunk length - 0.59mm.

Holotype: Worker; India, Kerala, Calicut University Campus; Sheela S, 8-12-1991 (Department of Zoology, University of Calicut).

Discussion: This species is unique among the species of *Tetramorium* in having peculiar shape for its gaster. Gaster is cordate as in figure 3. But in all other species gaster is not cordate.

Etymology: The species is named as *cordatus* to indicate its heart shaped gaster.

2. *Tetramorium keralensis* sp. nov. (Figs. 4-7)

Holotype worker: Head finely reticulate, including ventral side except mid dorsal line, occipital margin widely emarginate in full face view; mandible striate, masticatory margin with three acute apical teeth followed by two or three widely spaced small denticles towards base, basalmost part without teeth; clypeus convex with a strong median longitudinal carina and two feeble lateral carinae; anterior clypeal margin entire, arched, with an anterior flange; frontal area depressed but not clearly defined, frontal carinae distinct reaching up to occipital corners; posterior margin of frontal groove not distinct, scrobe finely punctate with two or three weak transverse striae just above antennal insertions and a few weak rugae backwards; eyes dorsal margin arched, ventral margin almost straight, lateral, prominent, placed on transverse mid line. Antennae 11–jointed, scape reaching upto occipital margin; 1st flagellar segment elongate, second subequal, 3rd and 4th transverse, 5-6 subequal, 7th elongate club thick, formed of apical three joints, terminal club segment longer than preceding two together (19 : 15); club distinctly longer than remaining flagellar segments together (34 : 29.5); longest hairs on frontal carinae shorter than diameter of eye.

Throax: dorsally finely reticulate, lateral portions not much strongly reticulate as dorsal; anteriorly broad, narrowing posteriorly; pro, meso and metanotum together forming a single convexity, sutures absent on dorsum of thorax, pronotum anteriorly produced into a short neck, anterolateral corners of pronotum rounded, not angular; propodeal spines long, stout, its length 2.6x its basal width and 6x distance between bases; spines curved slightly inwards but diverging from base, spine length : peduncle of petiole (9 : 6); propodeal spiracles large, situated just below base of spines (Fig. 4); metapleural lobes short, rounded, not acute at apex; legs slender, elongate, smooth at base, fore coxae shagreened, mid and hind tibiae with simple, weak spur.

Abdomen: Petiole and postpetiole finely minutely punctate, peduncle of petiole smooth, polished, petiole node with a few faint reticulations, peduncle of petiole curved in front, node quadrate, a little higher than long, dorsally longer than broad, a little longer than anterior penduncle (7 : 6); post petiole broader than long, rounded, globose, lower in a level than petiole, anterior and posterior faces of petiole almost parallel, above nearly convex. Gaster smooth, polished and shining, somewhat globose, junction of postpetiole and gaster except mid ventral portion with cross ridges, first tergite covering more than 3/4 its total length; sting with lamelliform appendage spatulate, projecting at an angle from shaft (Fig. 4).

Head, thorax, antennae, ,mandibles, legs and pedicel uniformly ferrugino–testaceous, gaster blackish brown; head and thorax with erect, pedicel and gaster with suberect, rather sparse, brownish yellow blunt setae; legs and antennae with appressed small hairs, femora with one or two erect silvery hairs of moderate length sub–basally.

Measurements: Total length–3.86mm; Head length - 0.80mm; Head width–0.74mm; Cephalic index–93; Eye diameter–0.18mm; Scape length–0.65mm; Scape index–88; Pronotal width–0.59mm; Alitrunk length–1.02mm.

Holotype: Worker, India : Kerala, Peruvannamuzhi, Sheela S, 17.1.1995 (DZCU)

Paratype: 1 worker, India : Kerala, Iravikulam, K. C. Gopi, 10.3.1994.

Discussion: This species comes close to *Tetramorium yerburyi* F. in the presence of long propodeal spines, and rounded pronotal angles. But it differs from *yerburyi* in (1) petiole and postpetiole punctate (Petiole and postpetiole rugose and reticulate in *yerburyi* (2) anterior and dorsal surfaces of petiole node not meet in a sharply defined right angle (anterior and dorsal surfaces of petiole node meet in a sharply defined right angle in *yerburyi* (3) size under 4mm (size above 4mm in *yerburyi*).

Etymology: The species name keralensis is given after its locality 'Kerala'.

3. *Tetramorium malabarensis* sp. nov. (Figs. 8-12)

Holotype worker: *Head* deeply rugose-reticulate; occipital margin broadly emarginate, mandibles finely striate, masticatory margin broad with three distinct, acute apical teeth and three or four inconspicuous irregular, small denticles towards base; clypeus sub-triangular, anterior margin entire, almost transverse with a flange in front; posterior margin of clypeus arched, three distinct longitudinal carinae on mid lobe of clypeus; frontal area depressed, frontal lobes short, wide apart, frontal carinae extending beyond eyes; scrobe distinctly defined by sculpture within it. Antennae 12 jointed, scape slender, all flagellar segments except first one and club transverse, club 3- jointed, longer than remaining flagellar segments together (14: 10); terminal club segment longer than preceding two together (8 : 6); eyes small, placed laterally on midline of head touching ventral margin of scrobe, elongately oval.

Thorax: Deeply sculptured like head, but median portion of propodeum smooth; evenly convex above, sides not margined, broad anteriorly, narrowing posteriorly; sutures absent; propodeal spines thick stout, acute at apex, prismatic, its length : distance between bases 8 : 6; in profile spine length 2.6x its basal width; spine length in profile 2.16x length of anterior peduncle of petiole; metasternal teeth triangular, acute at apex, legs smooth short, thick, tibiae and tarsi with a few very long erect hairs, length of some of those hairs on tarsi almost equal to length of basitarsus; on tibiae–length of tibiae 2.5x length of hairs on it.

Abdomen: except anterior peduncle deeply sculptured like head and thorax, anterior peduncle smooth short; nodes subequal in length, length of petiole 2.3x length of peduncle; petiole broader than postpetiole but not as broad as pronotum; height of petiole equal to its length, height of postpetiole more than its length. Gaster biconvex first tergite and sternite finely, longitudinally reticulate, remaining segments shagreened apically, first tergite covering more than 3/4 its total length, anterolateral angles of gaster almost angular but not projecting forward as tubercles or teeth; lamelliform, appendage triangular, projecting at an angle from shaft (Fig. 8).

Colour deep reddish brown, legs yellow, mandibles reddish yellow with masticatory margin black, antennae and legs reddish yellow, nodes of pedicel and gaster a little darker than head and thorax. Entire specimen covered with long, thin, erect, pale yellow hairs.

Measurements: Total length – 3.12mm; Head length – 0.74mm; Head width – 0.74mm; Cephalic index – 100; Eye diameter – 0.15mm; Scape length – 0.34mm; Scape index – 46; Pronotal width – 0.59mm; Alitrunk length – 0.89mm.

Holotype: Worker, India : Kerala, Calicut University Campus, Sheela S, 10.12.1991 (Department of Zoology, University of Calicut).

Discussion: This species comes between *Tetramorium rugigaster* Bolt. and *Tetramorium transversarium* R. It differs from *rugigaster* in that (1) anterolateral angles of gaster angular but not produced into tubercles or teeth (in *regigaster* anterolateral angles of gaster produced into fine tubercles) (2) first gastral tergite and sternite entirely finely reticulate (in *rugigaster* only basal half of first tergite and sternite regulose).

The species differs from *T. transversarium* R. in that (1) petiole node normal (petiole node enormously developed in *transversarium* (2) dorsum of first gastral tergite finely reticulate (basal 1/3 of first gastral tergite feebly rugulose and with traces of superficial punctation in *transversarium*).

Etymology: The species name *malabarensis* indicate its type locality.

4. *Tetramorium petiolatus* sp. nov. (Figs. 13-16)

Holotype worker : Head finely reticulate including ventral side, on ventral side sculpture not strong as on dorsal side and becoming feeble towards mid ventral line; reticulation within scrobe smaller than those of remaining parts; mandibles smooth polished with hair pits, masticatory margin with three acute teeth at apex followed by two denticles and then a bare area at base; clypeus anterior margin medially indented and depressed, no flange, mid lobe with three median carinae which extend backwards upto middle of head and branched beyond posterior clypeal margin; median carina not extending upto anterior margin, weak sublateral carinae also present on clypeus; frontal area triangular, shallow; frontal carinae distinct extending upto occipital corners; scrobe shallow, eyes large, prominent, lateral, almost round, situated almost on mid line, a little upwards. Antennae 12-jointed, scape slender reaching just below occipital corners, flagellar segments 2-7 transverse, F8, subequal F1 and club elongate, club 3–jointed, thick, longer than remaining flagellar segments together (19 : 16), terminal club segment longer than preceding two together (10:9).

Thorax: finely reticulate, reticulations on dorsal surface large, spaces within reticulation smooth, neck of pronotum punctate; with a short median transverse carina, anteriorly broad, on pronotum, a slight depression just below anterior margin (excluding neck) anterolateral corners angulate, sutures absent, margin of mesometanotal junction constricted, propodeal spines erect, not cylindrical, somewhat prismatic, pointing backwards and outwards, extreme tip pointing upwards, its length about 3x distance between its bases, and 2.5x basal width; 1.7x length of peduncle of petiole; metapleural lobes acutely pointed at apex; fore coxae shagreened remaining parts of legs smooth; femora medially swollen, spurs on mid and hind tibiae slightly pectinate.

Abdomen: Peduncle of petiole weakly punctate, nodes reticulate, sculpture on postpetiole weaker than that on petiole; petiole quadrate, convex above, posterior face a little higher than anterior face (Fig. 13); peduncle shorter than petiole node (6 : 10);

node height and length subequal; anterior and posterior faces sub-parallel, postpetiole rounded, lower in a level than petiole, anterior face sloping, petiole dorsally longer than broad, postpetiole transverse. Gaster smooth except few basigastral costulae, subglobose, first tergite covering more than 3/4 its length, anterior margin dorsoventrally with ridges.

Colour blackish brown, gaster darker than head and thorax; antennae, mandibles, legs, propodeal spines and apical margin of gastral tergites testaceous. Head, thorax, pedicel and abdomen with moderate amount of long and strong brownish yellow bristles, those on head and thorax, pedicel and abdomen with moderate amount of long and strong brownish yellow bristles, those on head and thorax erect, on pedicel suberect and on gaster subdecumbent; antennae and legs with abundant, short, decumbent hairs; femora with sparse long erect setae.

Measurements: Total length–4mm; Head length–0.93mm; Head width–0.93mm; Cephalic index–100; Eye diameter–0.24mm; Scape length–0.62mm; Scape index–67; Pronotal width–0.64mm; Alitrunk length–1.1mm.

Holotype: Worker, India : Kerala, Muthanga, Sheela. S, 7.10.1995. (Department of Zoology, University of Calicut).

Discussion: This species comes close to *Tetramorium pacificum* Mayr in the following characters : HW > 0.65mm, SL > 0.55mm, head rugosoreticulate, colour dark brown, T1 with basal costulae. But it differs from *pacificum* in that (1) in the shape of petiole node–in this new species postero–dorsal angle of petiole node a little above antero–dorsal but not much higher as in *pacifium* (2) eye larger in size than *pacificum* (0.18-0.21 in *pacificum*, 0.24 in new species) (3) cephalic index 100 (in *pacificum* 83-90) and scape index 67 (79-87 in *pacificum*).

Etymology: The species name '*petiolatus*' indicates the peculiar shape of the petiole.

5. *Tetramorium sentosus* sp. nov. (Figs. 17-20)

Holotype worker: Head anteriorly evenly rugulose, regulae diverging posteriorly and tending to be reticulate beyond level of posterior margin of eyes, posteriorly rugosoreticulate; genae and ventral portions of head longitudinally rugosoreticulate, scrobe with sculpture like that of nearby places; posterior margin of head transverse in front view, sides nearly straight, mandible closely longitudinally striate, masticatory margin with two acute apical teeth followed by two or three small teeth; clypeus with a narrow flange in front; a median strong carina accompanied by two pairs of weak carinae on either side present on mid lobe of clypeus; anterior margin entire, transverse, posterior clypeal margin arched, indistinct, frontal area very small, triangular; frontal lobes short, frontal carinae weak, extending well beyond eyes; posterior margin of scrobe faintly indicated, frontal carina reaching beyond eye by 1.25x length of eye, scrobes not distinctly marked; eyes elongate (Fig. 17) situated on mid transverse line, but in profile more close to mandibular base. Antennae 12–jointed, scape extending up to vertex, flagellar segments 2-8 transverse, 2-6 very short, club 3 jointed, terminal club segment longer than preceding two together (10 : 7) flagellum excluding club subequal to terminal club segment.

Thorax: Dorsally rugosoreticulate, sides rugulose, propodeum in between spines rugoso punctate; pronotum anteriorly transverse, sides not sharply pointed but giving a square shouldered appearance, in dorsal view margins straightly converging to propodeal spines; no sutures on thorax, episternum with a shallow diagonal depression, promesosternal separation faintly indicated, propodeal spines long, laterally compressed, directed backwards and upwards, tips acute and a little curved upwards, in dorsal view spines slightly diverging, distinctly longer than distance between its bases (7 : 5), length 2.3x basal width; propodeal spiracles situated below base of spines, almost at mid length of episternum, but towards posterior margin, metapleural lobes large, flat, shorter than propodeal spines, rounded at tip, subtriangular as in figure 17, length : basal width = 5 : 4; legs smooth, polished and shining, femora medially swollen, tibiae completely swollen, mid and hind tibiae with simple spurs.

Abdomen: Peduncle of petiole short smooth, petiole node rugosoreticulate with short ventral flange, post petiole weakly sculptured, peduncle subequal to metapleural lobes and shorter than propodeal spines; petiole massive, blocky, no constriction behind node, petiole height and length subequal, nearly convex above, anterior and posterior margins sub-parallel and vertical, post petiole shorter and a little lower than petiole, in dorsal view petiole length and breadth subequal and posteriorly broader than anterior side; post petiole in dorsal view distinctly broader than long, junction between post petiole and gaster with cross ridge. Gaster smooth polished and shining, anterior margin highly concave, anterolateral corners acute, projecting distinctly forward beyond posterior margin of post petiole; first tergite covering more than 3/4 its length.

Colour of head, thorax and pedicel brownish red, first tergite of abdomen excluding posterior margin dark reddish brown, posterior margin and remaining tergites reddish yellow; mandibles and antennae almost concolourous with head, legs reddish yellow; head, thorax, pedicel and gaster with brownish yellow, short, erect and suberect hairs, hairs abundant on gaster, legs with appressed very short hairs, tibiae with few long (as long as those on thorax) suberect hairs.

Measurements: Total length–2.16mm; head length–0.58mm; Head width–0.53mm; Cephalic index–91; Eye diameter–0.12mm; Scape length–0.40mm; Scape index–75; pronotal width–0.37mm; Alitrunk length–0.64mm.

Holotype: Worker, India : Kerala, Calicut University Campus, Sheela. S, 12.3.1995 (Department of Zoology, University of Calicut).

Discussion: This species comes close to *T. mixtum* F. in the following features (1) base of first gastral tergite concave behind post petiole and anterolateral corners of gaster prominent (2) mandibles striate in both species. But it differs from *mixtum* in (1) Anterolateral corners though projecting forward, not produced into blunt teeth or horn) (2) anterior margin of clypeus entire, not indented medially (in *mixtum* anterior clypeal margin slightly indented medially). The new species is smaller in size than *mixtum* .

Etymology: The species is named as *Sentosus* to indicate the peculiar metapleural spine.

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Spiders of the Genus *Cyclosa* Menge (Araneae : Araneidae) from Bangladesh

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Abstract: The genus *Cyclosa* Menge in Bangladesh is represented by five species, of which four, *C. bituberculata*, *C. elongata*, *C. tropica* and *C. yaginumai* are recongnised as new to science. The other species *C. confraga* (Thorell) appears new from the country. The new species are described and illustrated.

Keywords: New species, New record, *Cyclosa*, Araneae, Araneidae, Bangladesh.

Spiders of Bangladesh are poorly worked out group as compared to the works done in neighbouring countries like – India (Pocock, 1900; Tikader, 1970; Tikader, 1982; Tikader and Bal, 1980; Tikader and Bal, 1981; Tikader and Biswas, 1981), Burma (Thorell, 1895), Pakistan (Dyal, 1935), (Chang and Zhang, 1991; Yin, *et al.* 1990; Zhao, 1993,), Japan (Tanikawa, 1992a; Tanikawa, 1992b; Tanikawa and Ono, 1993; Yaginuma, 1986), Singapore (Koh, 1989) etc. Till date, works on Bangladesh spiders refers to Catling (1980), Chowdhury and Nagari (1981), Chowdhury and Pal (1984), Biswas, *et al.* (1993) and Okuma, *et al.* (1993).

We have recorded several spider species since 1988, of which the genus *Cyclosa* is found to be composed of five species. Of these, four, *C. bituberculata*, *C. elongata*, *C. tropica* and *C. yaginumai* are recognised as new to science and *C. confraga* (Thorell) as new record from the country. The new species are described and illustrated.

The types are at present in the collection of the Department of Zoology, Government P. C. College, Bagerhat, Bangladesh and will be deposited to the Museum of the Department of Zoology, University of Dhaka, Dhaka–1000, Bangladesh, in due course of time.

Abbreviations used : B. L. = Body length, C. L. = Carapace length, C. W. = Carapace width, A. L. = Abdomen length, A. W. = Abdomen width; B. A. R. I. = Bangladesh Agricultural Research Institute.

Genus : Cyclosa *Menge, 1866* (1866. *Cyclosa* Menge, Schrift. nat. Ges. Danzig. (N. F.), 1 : 73).

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Diagnosis : Body brown to dark-brown, elongate. Cephalothorax with anteriorly narrow cephalic region which in female U-shaped, raised with distinct cervical furrows; in male, cephalic region not much elevated and cervical furrows weak or almost absent. Eyes variably developed; posteromedians rather close and situated on a prominent tubercle; ocular quad wider in front. Legs long, slender and with hairs and small spines. Palpal patella of male with one large strong spines.

Abdomen with hump dorsally, these sometime paired laterally and unpaired medially, in some, with sigilla, variable in number and shape; epigyne having small, weak scape, may be straight, bent, pointed and circular.

Type–species : *Cyclosa conica* (Pallas).

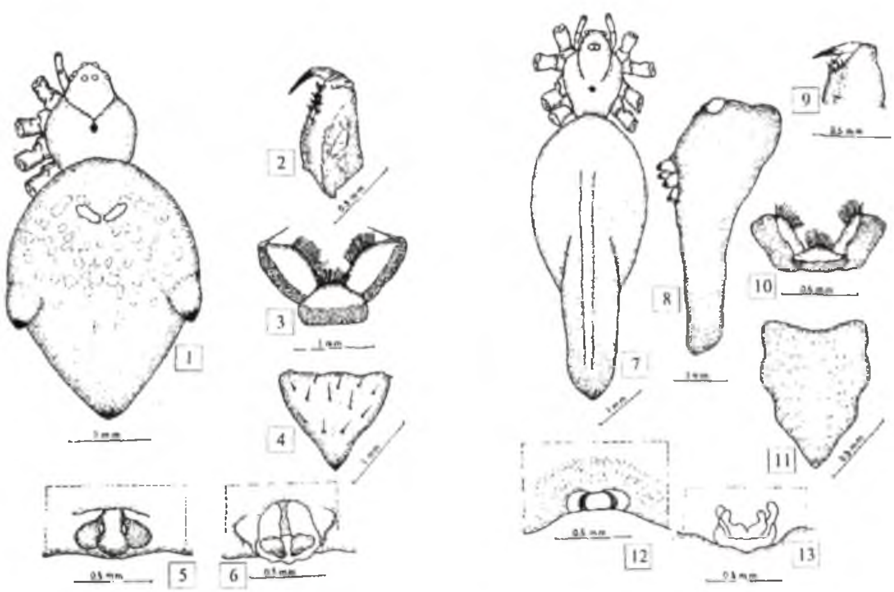
Distribution : AFRICA: AMERICA: ASIA: AUSTRALIA and EUROPE.

Key to the species

1. Abdomen posteriorly with a median tongue-like hump 2
   - Abdomen without any such hump 3
2. Median hump greatly elongate and broad; lateral hump absent; cervical furrows deeply distinct, cephalic region produced; sternum heart-shaped; cheliceral outer margin with 2 teeth; epigyne as in Fig. 12.
   .... *elongata* n. sp.
   - Median hump short and slender, lateral humps present; cervical furrows weak, cephalic region not produced; sternum 'V' -shaped; cheliceral outer margin with 3 teeth; epigyne as in Fig. 32
   .... *confraga*
3. Abdomen broadly triangular, with 2 large lateral tubercles; sternum triangular; cheliceral outer margin with 4 teeth; epigyne as in Fig. 5
   .... *bituberculata* n. sp.
   - Abdomen elongate, oval, without any such lateral tubercle; sternum never triangular; cheliceral outer margin atmost with 2 teeth 4
4. Abdomen posteriorly greatly produced, mid-longitudinally reticulate; cephalic region anteriorly bluntly pointed; sternum with white marginal patches, posteriorly blunt; cheliceral outer margin with 2 teeth; epigyne as in Fig. 18
   .... *tropica* n. sp.
   - Abdomen posteriorly not produced, devoid of any reticulation; cephalic region anteriorly rounded; sternum without any patch, posteriorly pointed; cheliceral outer margin without any teeth; epigyne as in Fig. 25
   .... *yaginumai* n. sp.

Cyclosa bituberculata n. sp. (Figs. 1–6)

Female : Cephalothorax brown black; legs brown; abdomen brownish with white spots. **Measurements** (Holotype, in mm) : B. L. = 5.20; C. L. = 1.50; C. W. = 1.10; A. L. = 3.70 and A. W. = 1.50; legs as in Table 1.



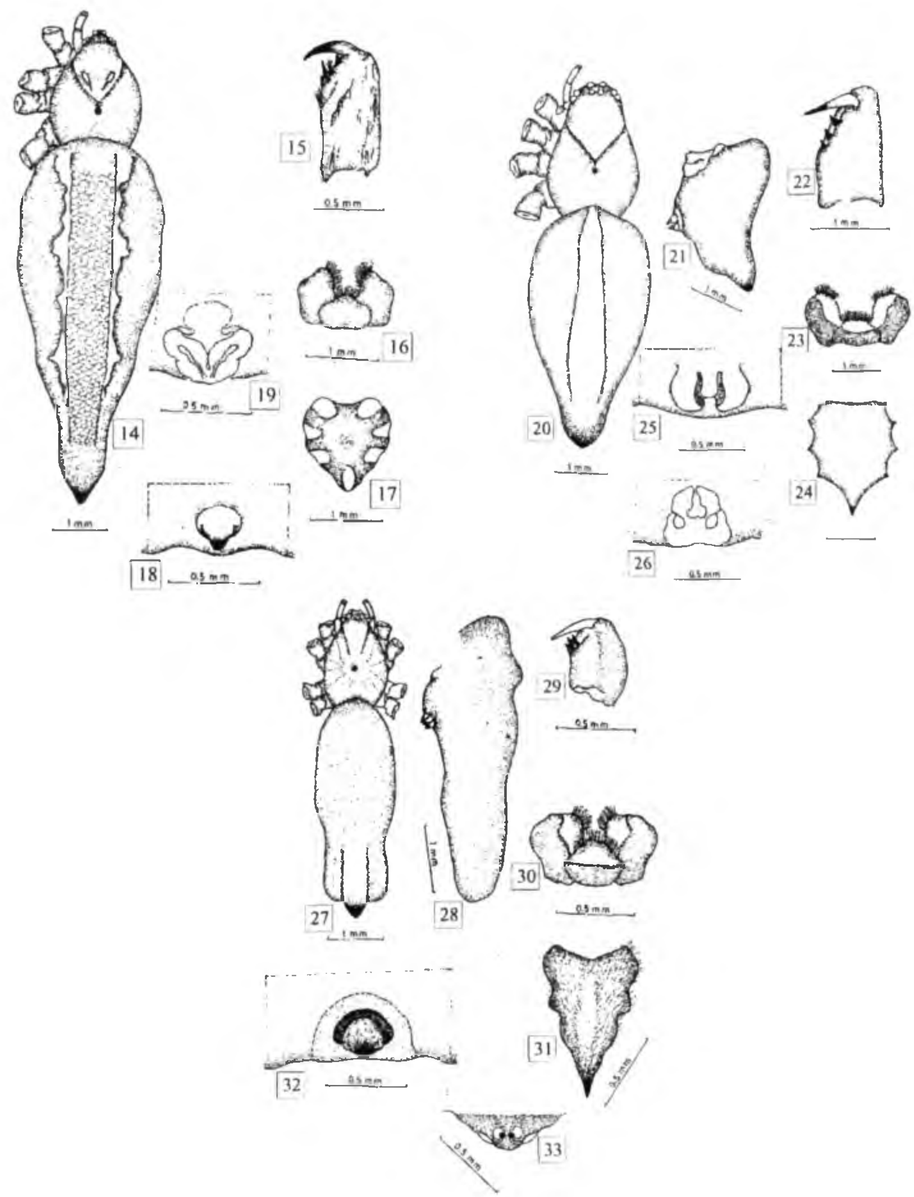
Figs. 1–6. 1. *Cyclosa bituberculata* n. sp. (female, dorsal view); 2. Chelicerae; 3. Maxillae and Labium; 4. Sternum; 5. Epigynum; 6. Internal genitalia.
Figs. 7 – 13. 7. *Cyclosa elongata* n. sp. (female, dorsal view); 8. Abdomen (lateral view); 9. Chelicerae; 10. Maxillae and labium; 11. Sternum; 12. Epigynum; 13. Internal genitalia.

Cephalothorax : broad posteromedially, anteriorly abruptly narrowed, longer than wide, clothed with fine hairs; cephalic region raised, strongly convex, with deeply distinct cervical furrows; eyes pearly-white, similar; anterior row strongly recurved, posterior row slightly procurved; anteromedians slightly larger than posertomedians and situated on protuberance, lateral eyes close; ocular quad rectangular, longer than wide; chelicerae brown, long, anteriorly narrowing, each of inner and outer margins with 4 teeth (Fig. 2); maxillae brown, longer than wide, boat-like, anteriorly scopulate (Fig. 3); labium brown, broad basally, anteriorly narrowing and scopulate (Fig. 3); sternum brown, triangular, anterior margin more or less straight and posteriorly bluntly pointed, clothed with sharp spines (Fig. 4); legs long and slender, with brown bands, clothed with hairs and spines; leg formula and measurements : 1423

Table 1. Length of legs of female (♀) holotype of *Cyclosa bituberculata* n. sp. (in mm).

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.0/2.0	0.9/0.9	1.5/1.5	1.3/1.3	0.8/0.8	6.5/6.5
II	2.0/2.0	0.8/0.8	1.0/1.0	1.0/1.0	0.7/0.7	5.5/5.6
III	1.5/1.5	0.6/0.6	1.0/1.1	0.9/1.0	0.5/0.5	4.5/4.8
IV	2.1/2.1	0.5/0.5	1.4/1.3	1.1/1.1	0.6/0.7	5.7/5.7

Abdomen : rhomboid, anteriorly wide, posteriorly produced and pointed, with 2 oval marginal humps and clothed with hairs (Fig. 1); ventrally pale with white patches,



Figs. 14 –19. 14. *Cyclosa tropica* n. sp. (female, dorsal view); 15. Chelicerae; 16. Maxillae and labium; 17. Sternum; 18. Epigynum; 19. Internal genitalia.
Figs. 20 – 26. 21. *Cyclosa yaginumai* n. sp. (female, dorsal view); 21. Abdomen (lateral view); 22. Chelicerae; 23. Maxillae and labium; 24. Sternum; 25. Epigynum; 26. Internal genitalia.
Figs. 27 – 33. 27. *Cyclosa confraga* (Thorell) (female, dorsal view); 28. Abdomen (lateral view); 29. Chelicerae; 30. Maxillae and labium; 31. Sternum; 32. Epigynum; 33. Internal genitalia.

clothed with hairs; epigyne and internal genitalia as in Figs. 5–6.

Male : Unknown.

Holotype : ♀, Barisal, 20. X. 1993, Coll. V. Biswas.

Paratype : 1 ♀, otherwise data same as for the holotype.

Distribution : BANGLADESH : Barisal.

Etymology : The species is named because of the presence of 2 (two) lateral tubercles on the abdomen.

Remarks : The new species stands distinct because of the rhomboid abdominal dorsum, with 2 marginal humps, very typical epigyne and triangular sternum from the other congeneric species known from India (Tikader, 1982); (Yaginuma, 1986), Japan (Shinkai and Takano, 1984; Chang and Zhang, 1991).

***Cyclosa elongata* n.sp.**

(Figs. 7–13)

Female : Cephalothorax and abdomen blackish brown; abdomen dorsally with whitish band; legs yellow with brown bands. **Measurements** (Holotype, in mm) : B. L. = 5.40; C. L. = 1.50; C. W. = 1.20; A. L. = 3.90; A. W. = 1.30; legs as in Table 2.

Cephalothorax : elongately oval, medially wide, narrowed at both ends, anteriorly produced and truncate; cephalic region raised, strongly convex; cervical furrows deeply distinct; thoracic region posteromedially with a deep brown fovea; eyes pearly-white, with black basal bands; both anterior and posterior rows strongly recurved; posteromedians larger and close; anteromedians placed on the lateral margins of the protuberance; lateral eyes close; ocular quad rhomboid; chelicerae brown, elongate, nearly parallel-sided, each of inner and outer margins with 2 teeth (Fig. 9); maxillae pale brown, elongate, scopulate anteriorly (Fig. 10); labium brownish, small, medially wide and anteriorly bluntly pointed and scopulate (Fig. 10); sternum brown, heart-shaped, anteriorly wide and concave, posteriorly narrowing, clothed with small spines (Fig. 11); legs long, slender, clothed with spines; leg formula and the measurements : 1423

Table 2. Length of legs of female (♀) holotype of *Cyclosa elongata* n. sp. (in mm).

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.0/2.0	0.8/0.8	1.5/1.5	1.2/1.2	0.8/0.8	6.3/6.3
II	2.0/2.0	0.8/0.8	1.0/1.0	1.0/1.0	0.6/0.6	5.4/5.4
III	1.0/1.0	0.5/0.5	1.0/1.0	0.9/0.9	0.5/0.5	3.9/3.9
IV	2.0/2.0	0.5/0.5	1.2/1.2	1.3/1.3	0.6/0.6	5.6/5.6

Abdomen : very long, anterior half elongately oval, posterior half produced and tongue-like (Figs. 7–8); medially with a longitudinal, white band, clothed with white hairs and brown spines; ventrally pale, anteromedially with spinnerets on the margin; epigyne and internal genitalia as in Figs. 12–13.

Male : Unknown.

Holotype : ♀, Mymensingh, 18. X. 1992, Coll. V. Biswas.

Paratypes : 1♀, Bagerhat, 19. X. 1993, Coll. V. Biswas; 2♀, Rangpur, 12. IV. 1991, Coll. V. Biswas.

Distribution : BANGLADESH : Bagerhat, Mymensingh and Rangpur.

Etymology : The species is named due to the elongate nature of the abdomen.

Remarks : The closest ally of the species appears to be *Cyclosa bifida* (Doleschall) (Tikader, 1982). Reasons for recognising the species as new to science are –

1. margins of abdominal dorsum discontinuous; posterior half originates medially from the distal region of anterior half of abdominal dorsum; abdomen dorsally with a white longitudinal band.
2. typical epigyne and
3. cephalothoracic radii absent.

Cyclosa tropica n. sp. (Figs. 14–19)

Female : Cephalothorax and abdomen brown black; abdomen dorsally with white bands; legs yellow with brown bands. **Measurements** (Holotype, in mm) : B.L. = 5.20; C.L. = 1.50; C.W. = 1.30; A.L. = 3.70; A.W. = 1.40; legs as in Table 3.

Cephalothorax : elongately oval, posteriorly wide, anteriorly narrowed and pointed, clothed with black hairs; dorsally with 2 white eye like markings; cephalic region elevated; cervical furrows deeply distinct; thoracic region with a deep central fovea; eyes pearly-white, basally with black patch; anterior row strongly and posterior row faintly recurved; anteromedians more widely placed, on a protuberance, posteromedians close; lateral eyes close; ocular quad posteriorly narrowed, longer than wide; chelicerae brown, elongate, parallel-sided, strong, each of inner and outer margins with 3 and 2 teeth (Fig. 15); maxillae brown, small, longer than wide, anteriorly narrowed and scopulate (Fig. 16); labium brownish, wider than long and anteriorly scopulate (Fig. 16); sternum brownish, typically heart-shaped, anteriorly broad and posteriorly narrowed, with white patches (Fig. 17), clothed with hairs; legs long and slender, yellow with brown bands; leg formula and the measurements : 4132

Table 3. Length of legs of female (♀) holotype of *Cyclosa tropica* n. sp. (in mm).

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.5/2.5	0.6/0.7	1.5/1.5	1.5/1.5	0.8/0.8	6.9/7.0
II	1.5/1.5	0.4/0.4	1.0/1.0	1.0/1.0	0.5/0.5	4.4/4.4
III	2.0/2.0	0.5/0.5	1.5/1.5	1.0/1.0	0.5/0.5	5.3/5.3
IV	3.2/3.2	1.0/1.0	1.8/1.8	1.5/1.5	0.8/0.8	8.3/8.3

Abdomen : long, anteriorly wide, posteriorly narrowed and produced, with a pointed tip; dorsum decorated with black and white longitudinal bands; ventrally black, with white patches, clothed with hairs; epigyne and internal genitalia as in Figs. 18–19.

Male : Unknown.

Holotype : ♀, Bagerhat, 18. IV. 1993, Coll. V. Biswas.

Paratype : 1♀, B.A.R.I., Jessore, 12. X. 1992, Coll. V. Biswas.

Distribution : BANGLADESH : Bagerhat, Jessore.

Etymology : The species is named because of its distribution in the tropical region.

Remarks : The new species may be related to its closest ally *C. bifida* (Doleschall) (Tikader, 1982) but stands distinct in having anteriorly narrowed cephalothorax, abdomen anteriorly broad, caudal process long and pointed. sigilla absent, epigynal scape short and bluntly pointed. None of the other *Cyclosa* species are known to possess such combination of characters (Chang and Zhang, 1991; Zhao, 1993; Koh, 1989; Shinkai and Takano, 1984; Yaginuma, 1986).

Cyclosa yaginumai n. sp. (Figs. 20–26)

Female : Cephalothorax brown black; abdomen brown with white scaly reticulation. **Measurements** (Holotype, in mm) : B.L. = 5.20; C.L. = 1.40; C.W. = 1.20; A.L. = 3.80; A.W. = 1.30; legs as in Table 4.

Cephalothorax : elongately oval, posteriorly broad, anteriorly narrowing; cephalic region raised, convex, with deeply distinct cervical furrows; thoracic region medially with a deep central fovea; eyes pearly-white; both anterior and posterior rows strongly recurved; anteromedians more wider and slightly larger than posteromedians; lateral eyes close; ocular quad posteriorly narrowed, slightly wider than long; chelicerae medially broad, inner margin with 3 pointed teeth (Fig. 22); maxillae brown, boat-like, anteriorly broad and scopulate (Fig. 23); labium brown, basally broad, wider than long and anteriorly scopulate (Fig. 23); sternum brown, broad, heart-shaped, pointed posteriorly (Fig. 24); legs long, with sharp hairs and spines; leg formula and the measurements : 2134

Table 4. Length of legs of female (♀) holotype of *Cyclosa yaginumai* n. sp. (in mm).

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	1.1/1.1	0.5/0.5	1.0/1.0	1.0/1.0	0.7/0.7	4.3/4.3
II	1.5/1.5	0.5/0.5	1.0/1.0	1.0/1.0	0.5/0.5	4.5/4.5
III	1.0/1.0	0.5/0.5	1.2/1.2	0.9/0.9	0.4/0.4	4.1/4.1
IV	0.9/0.9	0.4/0.4	1.0/1.0	0.9/0.9	0.4/0.4	3.6/3.6

Abdomen: elongately oval, broad anteriorly, posteriorly narrowed and produced as bluntly pointed tip; dorsum decorated with a longitudinal white reticulate band; anterior humps more evident in profile; ventrally pale-brown, clothed with hairs; epigyne and internal genitalia as in Figs. 25–26.

Male: Unknown.

Holotype: ♀, S.Park, Dhaka, 4. III. 1992; Coll. V. Biswas.

Paratypes: 2♀, otherwise data same as for the holotype.

Distribution : BANGLADESH : Dhaka.

Etymology : The species has been named after late Professor Takeo Yaginuma, a famous Arachnologist of Japan.

Remarks : The species appears close to *Cyclosa tropica* n. sp. but typical epigyne, much short caudal process, abdomen anteriorly produced, overhanging the cephalothorax, cephalothorax anteriorly broad and truncate, outer margin of chelicerae without any teeth, general shape of maxilla, labium, sternum etc. justify its recognition as new to science.

Cyclosa confraga (Thorell) (Figs. 27–33) 1892. *Epeira contraga* Thorell, Bull. Soc. ent. Intal., 24 : 239.

Material examined: 4♀, Bagerhat, 12. VIII. 1993, Coll. V. Biswas; 2♀, Fardipur, 19. IX. 1992, Coll. V. Biswas; 1♀, Kustia, 8. XII. 1993, Coll. V. Biswas; 10+, Manikgang, 15. X. 1992; Coll. V. Biswas; 2♀, Rajshahi, 15. III. 1992, Coll. V. Biswas; 2♀, Rangpur, 12. XI. 1993, Coll. V. Biswas; 1♀, Sylhet, 28. XII. 1993, Coll. V. Biswas.

Distribution: BANGLADESH : Bagerhat, Faridpur, Kustia, Manikgang, Rajshahi, Rangpur, Sylhet; INDIA; BURMA; JAPAN; KOREA (Tikader, 1982).

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The authors are grateful to Dr. S. C. Mazumder, Scientist-B, Sundarban Retd Research statin, Zoological Survey of India, Canning West Bengal, for confirming the identity of the species and the Head, Department of Zoology, University of Calcutta, for providing Laboratory facilities.

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Oribatid Mites From Coconut Palm–5. A New Species of *Scapheremaeus* Berlese, 1910 (Acari: Cymbaeremaeidae) From Kerala, India

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Abstract: A new oribatid mite, *Scapheremaeus nuciferosa* sp.nov. collected from the green foliage, particularly on lower surface of the leaflets, of coconut palm and also the leaves of *Chromolaena odorata* is described and illustrated. The genus is reported for the first time from India.

Keywords: Cymbaeremaeidae, *Scapheremaeus*, Coconut, *Chromolaena*

INTRODUCTION

The genus *Scapheremaeus* was erected by Berlese (1910) with *S. patella* as the type for the genus. During the succeeding years, new additions to the genus were made from different countries like Argentina, Bolivia, Surinam, Algeria, Fiji Islands, New Zealand, Mauritius, Philippines etc. The members of this genus are usually sluggish and their known habitats include soil, dead and dry leaves, moss, branches of trees, leaves of plants etc. The present species also enjoys an arboreal habitat, on fresh leaves of coconut palm and *Chromolaena odorata*.

Scapheremaeus nuciferosa sp. nov.(Figs 1–4)

Colour: Dark brown to black.

Measurements: Length: 319 μm (Range: 293–344 μm)

Width: 166 μm (Range: 140–204 μm)

Prodorsum:(Fig. 1) Prodorsum flat, roughly conical, broader than longer with a blunt rostrum. Seta *ro* (Fig. 1a) inwardly directed, feebly barbed and measures 27 μm. Lamellae narrow, bar shaped, originate slightly above the bothridia and run forward to a short distance giving off two branches anteriorly, one exterior and the other interior; at the tip of the latter arises the weakly barbed seta *le* (Fig. 1b) which measures 7

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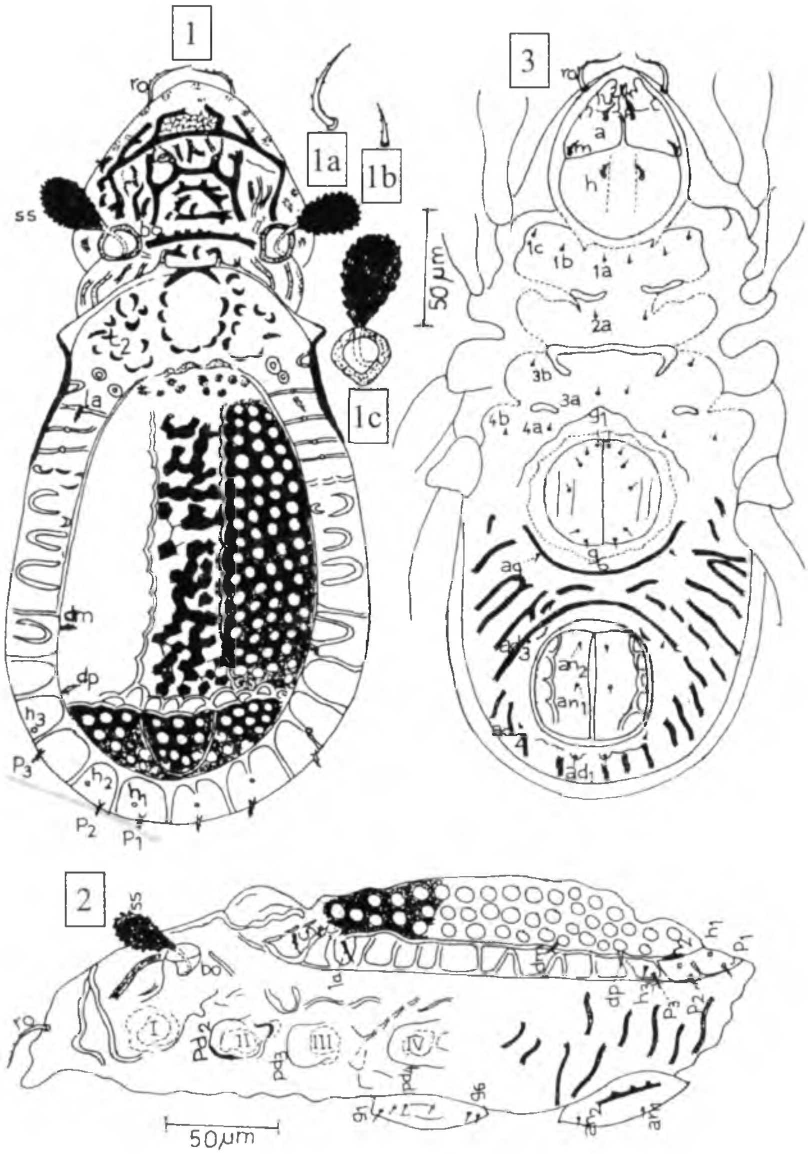


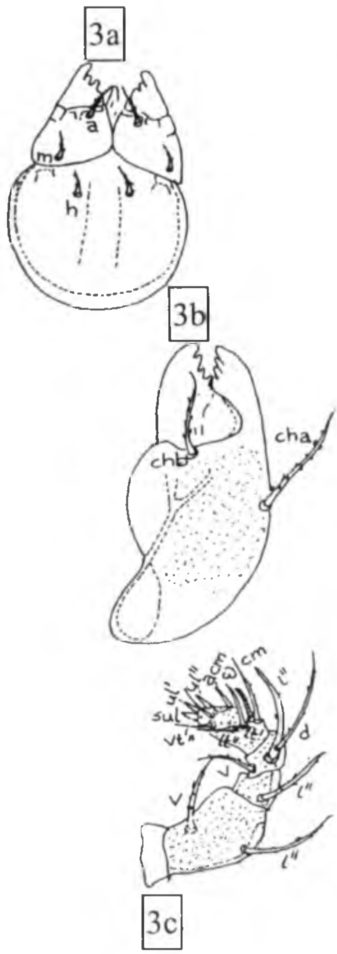
Fig. 1: Dorsal region; (1a) rostral seta; (1b) lamellar seta; (1c) sensillus
Fig. 2: *Scapheremaeus nuciferosa* sp. nov. – Lateral region

μm in length; the inner branches of the two sides connected by a median translamella. The space between the lamellar ridges and the bothridia occupied by three transverse ridges, the anterior one with four branches, the median one with posterolateral ramification and the posterior one with several minute anterior projections. Just above the anterior border of each bothridial cup a narrow band present which diverges lat-

erally and then turns anteromedially to meet its fellow of the opposite side; above the level of the lamellar and translamellar ridges, two branches arise from this band anteriorly, of which the inner one of both sides curved and extend forward to encircle the reticulations present inside, the outer branch simple and stops abruptly. The inter-space between the lamellar ridge and the outer branch provided with two to three faint discontinuous band like structures and aggregates of punctuations. The space between the translamella and the median band also possesses two lateral simple bands and a median bifurcated band. The extreme lateral borders of the prodorsum provided with simple to curved faint bands and isolated groups of punctuations. The anterior and anterolateral regions of the rosturm show the presence of groups of punctuations aggregated together irregularly. The region of the prodorsum lying posterior to the bothridia and immediately above the dorsosejugal suture provided with several less scleerotised bands of varying size. Interlamellar setae and exobothridial setae absent. Bothridial cup (*bo*) with wide opening. Sensillus (*ss*) (Fig. 1c) short stalked with a large club shaped black head.

Notogaster: (Fig. 1)

Notogaster elongated with an almost circular posterior border. Dorsosejugal suture complete and slightly convex giving off two median short ridges anteriorly. Humeral region of the notogaster provided with moderately developed humeral process; anterolateral borders of the notogaster below the humeral process thickened. Just below and touching the dorososejugal suture an oval shaped lenticulus present with interrupted boundary; on either side of the lenticulus several semilunar sclerotised structures arranged in an irregular manner. The region of the notogaster lying behind the lenticulus and associated structures greatly ornamented and differentiated into a marginal zone and a median zone, the two being clearly separated from each other by a distinct boundary. The marginal zone at the extreme anterior region provided with a pair of circular thickening and a 'U' shaped structure on each side behind which rib like structures present at the middle region of the marginal zone and simple bar shaped structures radiate to the periphery at the extreme posterior and posterolateral regions of the marginal zone. The median zone further differentiated into an innermost depressed or concave area containing irregularly arranged sclerotised polygonal structures with intermittent connections forming semicircular, squarish to shapeless articulations; outer to the concave innermost area on either side, elevated sclerotised areas present with thick integument interspersed with clearly spherical foveolae giving a pitted appearance; the inner and outer regions of the median zone clearly demarcated by a thick wavy vertical line running from the anterior region of the median zone to about 3/4 of its length which later unites with a chain of reticulations running horizontally in the median zone; the posterior region of the median zone separated from the remaining part by the horizontal chains of reticulations; posteriorly the middle zone further divided into inner and outer regions, all equally elevated and formed of thick integument interspersed with round foveolae; the inner region separated from the outer region by a 'U' shaped boundary which anteriorly unites with the horizontal chain of reticulations; at the extreme posterior part of the median zone the integument less sclerotised; the anterior most region of the median zone with irregularly arranged groups of punctuations. Ten pairs of small, spiniform, barbed setae distributed on the notogaster (Fig. 1), eight pairs inserted on the marginal zone and only two pairs on the median zone;



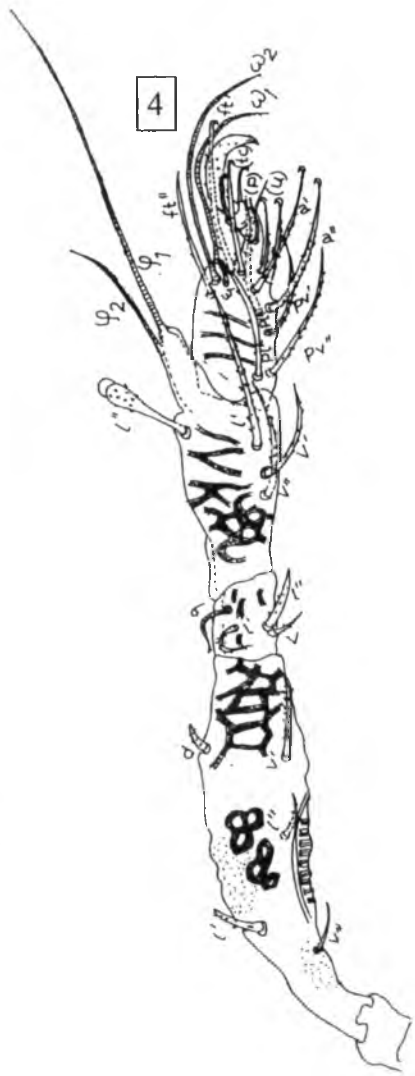


Fig. 3: *Scapheremaeus nuciferosa* sp. nov. – Ventral region; (3a) gnathosoma; (3b) chelicera; (3c) pedipalp
Fig. 4: Leg I

setae h_1, h_2 and h_3 in the holotype fallen and represented only by their alveoli.

Lateral region: (Fig. 2)

In lateral view, genal tooth appears pronounced. Tutorium not present. Pedotecta II, III and IV detected.

Ventral region: (Fig. 3)

Gnathosoma: (Fig. 3a)

Mentum separated from the genae by a slightly arched labiogenal articulation. The infracapitulum smooth and without any ornamentation. Mentum large and genae small. Rutellum (*ru*) with three blunt, poorly sclerotised notches. All the three pairs of infracapitular setae (*h*,*m* and *a*) barbed, *a* slightly longer than *h* and *m*. Chelicerae (Fig. 3b) short, broad and with porose surface; each chelicera with four to five pairs of blunt, poorly sclerotised teeth; setae *cha* and *chb* barbed, the former longer than the latter; pedipalps (Fig. 3c) five segmented with a setal formula of 0–2–1–3–10; all segments except trochanter appear porose; palpal tarsus with a single solenidion ω and four eupathidia, *acm* not associated with the solenidion, *cm* with very faint barbs, others smooth.

Epimeral region: Apodemata II, III and the sejugal apodeme present, the latter of both sides continuous with each other medially forming a cap like structure. Epimeral setal formula 3–1–2–2, all setae very short and smooth.

Genital and anal regions: Genital plates bordered anteriorly by an arched, wavy ridge and posteriorly by a highly sclerotised concave ridge. Genital plates almost circular with six pairs of smooth setae, g_1 longer than the others, g_4 and g_5 widely separate; longitudinal striations present on the genital plates. One pair of smooth, short aggenital setae (*ag*) located posterolateral to the genital plates just below the concave ridge. Anal plates elongated and more or less rectangular in appearance carrying a pair of wavy sclerotised ridges close to their outer margins; each anal plate possesses a pair of short, smooth setae, an_1 inserted at the middle and an_2 inserted at the anterior region of the anal plate. Three pairs of short adanal setae arranged on the adanal area, just outer to the anal plates; ad_1 post-anal in position with prominent tubercles, ad_2 and ad_3 para-anal. Fissure *iad* oblique, placed close to the anal plates, slightly above ad_3. The lateral region between genital plate and anal plate marked by curved, branched to broken rides.

Legs: Legs tridactylous possessing prominent heterodactyly. Polygonal reticulations and branched sclerotised ridges present on all leg segments. Chaetotaxy of leg I(Fig. 4) 0–5–4–6–16. Trochanter I very small and devoid of any setae. Femur I elongated, stout and carries porose areas, reticulations and a ridge, among the five setae of femur I, two (*d* and *l'*) thick, blunt and barbed, *v''* smooth. Genu I short carrying a thin curved solenidion σ and three barbed setae, *l''* comparatively more thick. Tibia I with a distal large protruberance for the insertion of the solenidia φ_1 and φ_2, the latter very thin and the former more thickened and greatly elongated, *l''* large and brush like. Tarsus I expanded proximally and very narrow distally carrying a pretarsus and three claws, the median claw very thick, large and spined and the lateral claws very thin, smooth and resemble ordinary setae, two solenidia (ω_1 and ω_2) and a famulus ε present on the tarsus, most of the tarsal setae curved apically with slightly swollen apex and provided with short barbs.

Materials examined:

Holotype: ♂ ;paratypes : 7 ♂♂ and 3 ♀♀ collected from the foliage of coconut palm, Calicut University Campus, Kerala, India on 2.2.84

Remarks:

The present new species, *S. nuciferosa* agrees with the Philippine species, *S. arboreus* described by Corpuz-Raros (1979) in the nature of the prodorsal and notogastral setae and in some of the ornamentation of the prodorsum and notogaster. But the following characters clearly differentiate *S. nuciferosa* from *s. arboreus*:

1. Presence of ten pairs of notogastral setae
2. Absence of the sclerotic ridge on the prodorsum
3. Discontinuous nature of the lenticulus and associated structures
4. Nature of ornamentation of the median zone and its further differentiation
5. Possession of an epimeral setal formula of 3–1–2–2 and
6. Presence of striations and curved ridges on the genital and anal plates respectively.

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Short Communication

Base–line data on the isoenzyme profile of fleas *Xynopsylla cheopis* and *X. astia*

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Abstract: Base–line data of isoenzymes profile of flea *Xynopsylla cheopis* and *X. astia* was determined. Marked differences were noticed in esterases, isocitrate dehydrogenase, malate dehydrogenase and octanol dehydrogenase enzymes. These enzyme system worked will with the fleas species, hence it can be used to resolve subspecies complexes.

Keywords: *Xynopsylla cheopis, X. astia,* Isoenzymes.

In Indian subcontinent *Xynopsylla cheopis* (Rothschild) and *X. astia* (Rothschild) are considered the efficient vector of zoonotic plague (Pollitzer, 1954; W.H.O, 1970; Renapurkar, 1990). Of these two flea vectors, *X. cheopis* predominates the commensal rodents and *X. astia* predominates on peridomestic and wild rodents (Renapurkar, 1990) . Though these tow species are well distinguished on the basis of morphological characters, no biochemical makers have been worked out so far to identify them. The present communication reports the isozyme profile of these two species.

Fleas were obtained from the colonies of *X. cheopis* & *X. astia* maintained since 1988 at the Institute of Vector Control and Zoonoses, Hosur, Tamil Nadu state. These were homogenised in homogenising solutions with the help of mortar and pestle and centrifuged at 4000 x g for 5 minutes. Polyacrylamide gel electrophoresis was performed on these homogenates in native conditions. The protocol followed for lactate dehydrogenase, malate dehydrogenase, glucose 6-phosphate dehydrogenase, isocitrate dehydrogenase, general esterases and octanol dehydrogenase was same as described by Munstermann (1979).

Results showed that there was difference in the isoenzyme profile of both the species. However, marked difference were noticed in esterases, isocitrate dehydrogenase, malate dehydrogenase, and octanol dehydrogenase (Fig. 1.). Isoenzyme parameters can be used to distinguish these species in peculiar situations where identification can not be made using morphological characters on live fleas. In addition, the enzyme system can be used to resolve subspecies complexes in these fleas.

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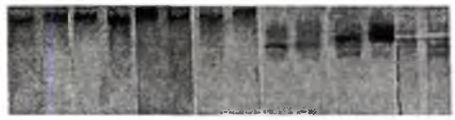

Fig. 1. Isoenzyme profile of homogenates of flea strains: Lactate dehydrogenase [Lane 1] *X.astia* [lane 2] *X. cheopis*; Malate dehydrogenase [lane 3] *X.astia* [lane 4] *X. cheopis*; Glucose 6-phosphate dehydrogenase [Lane 5] *X.astia* [lane 6] *X. cheopis*; Isocitrate dehydrogenase [Lane 7] *X. astia* [Lane 8] *X. cheopis*. Esterase-A [Lane 9] *X. astia* [Lane 10] *X. cheopis*; Esterase-B [Lane 11] *X. astia* [lane 12] *X. cheopis*; Octanol dehydrogenase [lane 13] *X. astia* [lane 14] *X. cheopis*.

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Short Communication

Influence of Host Plants on the Activity of the Digestive Enzymes in *Helicoverpa armigera* (Hubner)

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Abstract: Activity of digestive enzymes in *H. armigera* (Hubner) was studied when fed on different food plants. *H. armigera* larvae when fed on Bengal gram strongly stimulate amylase, invertase and protease enzyme as compared to cotton, tomato, Bhendi and sunflower. Lipase activity was more when fed on sunflower as compared to red gram, Bengal gram, tomato and Bhendi.

Keywords: *Helicoverpa armigera*, amylase, invertase, protease, lipase.

INTRODUCTION

The American bollworm, *Helicoverpa armigera* (Hubner) is one of the most voracious pests, which heavily attack field, vegetable and oilseed crops. The larval lifespan and its growth and development differ widely according to the types of food consumed. The nutritional need and the knowledge of the functional organization of digestive system of insect may be useful in devising new measures of control. Furthermore, information might be useful to understand how this insect adopts to its natural food. In general, soluble carbohydrates and proteins are very efficiently utilized by the insect and most of the species derived the largest share of their nourishment from these nutrients (Ishaaya, *et al.* 1971,) while the utilization of these nutrients form the available food plants depends on the digestive enzymes. Despite ample information concerning biochemical properties of digestive enzymes. (Ishaaya, *et al.* 1971,), relatively little is known about the influence of different host plants on the activity of digestive enzymes of *H.armigera*. According to Hori (1969) certain plant compounds can activate or inhibit digestive enzymes, it can be assumed that the latter, in turn, affect digestion and food utilization.

Since protease, amylase, invertase and lipase activity are of great importance in the digestion of food, the experiment was conducted to determine their activity in *H.armigera* when fed on different host plants.

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Third instar larvae (28 to 30 mg weight) of *H.armigera* reared on semi-synthetic diet were used for this study. These larvae were starved for 12 hours and then released on different host plants, viz. cotton, tomato, Bhendi, red gram, Bengal gram and sunflower and allowed them to feed upto 48 hours. Then the larvae were removed from the host and allowed to starve for 12 hours. The alimentary canals were dissected from three such larvae and crushed in ice cold 0.2 ml phosphate buffer (pH 8–9). The homogenate was then centrifuged at 10,00 Xg for 15 minutes in a refrigerated centrifuge. The supernatant was used as enzyme source.

Determination of enzyme activity

i) Amylase activity was determined based on the method of Ishaaya and Swiriski (1970) and as described by Sadasivam and Manickam (1992) using 3,5-dinitrosalicyclic acid reagent;

ii) For determination of invertase activity, the method of Ishaaya and Swiriski (1970) was followed;

iii) Protease activity was determined UV spectrophotometrically by following the method as suggested by Ishaayaa and Swiriski (1970);

iv) Lipase activity was determined by method described by Sadasivam and Manickam (1992).

The enzyme activity observed in larvae which fed on various host plants is presented in Table–1.

Table 1. Influence of different host (food) plants on activity of digestive enzymes in *H. armigera*

Food Plants	Food consumed (g)	Enzyme activity (X ± SD) Amylase μg/g of gut tissue	Invertase μg/g of gut tissue	Protease OB units/g of gut tissue	Lipase activity/g of gut tissue
Cotton	1.27	127.22 ±3.68	1.75±0.04	64.43±3.13	1674.33±13.07
Tomato	1.25	92.30±2.12	1.58±0.03	0.65±0.04	643.67± 4.64
Sunflower	1.87	101.23±4.72	1.79±0.04	332.95±3.97	1960.33± 7.41
Red Gram	1.24	132.86±4.49	1.56±0.04	343.67±4.86	955.33± 8.99
Bengal Gram	2.16	217.56±3.56	2.76±0.03	394.00±6.08	1253.00±12.03
Bhendi	1.20	123.15±4.97	1.32±0.03	28.43±1.12	794.67±10.96

Amylase activity

The activity of amylase in terms of production of maltose/g of gut tissue/30 min is more in the larvae which fed on Bengal gram (217.56) followed by red gram (132.86). However, less activity was found in the larvae fed on tomato (92.30).

Invertase activity

Invertase activity was expressed in terms of production of glucose liberated/g of gut tissue/hr. The activity of enzymes was more in larvae fed on Bengal gram (2.76) followed by sunflower (1.79) and low in Bhendi (1.32).

Protease activity

The activity of protease enzyme was expressed in OD units/g of gut tissue. The enzyme activity was found to be high in larvae fed on Bengal gram (394.00 OD units/g of gut tissue) followed by red gram and low activity in larvae fed on Bhendi.

Lipase activity

The activity of lipase enzyme was found to be more in the larvae fed on sunflower (1960.33 lipase units/g of gut) followed by cotton (1674.33) and low on tomato (643.67).

Digestive enzymes are vital determinants for growth and survival of herbivores. The food quality regulates and influences the production of digestive enzymes. An attempt was made to study the activity of digestive enzyme because of the type of food was considered as one of the major causative factor for the stimulation of enzyme activity.

The activity of digestive enzymes differ widely with the type of food given to phytophagous larvae (Soottoo and Fraenkel, 1966). The activity of amylase appeared to be high when larvae fed on Bengal gram, red gram and cotton as compared to tomato, Bhendi and sunflower. The activity of invertase enzyme was found to be more in larva fed on Bengal gram and sunflower which digest and utilise sucrose. The lipase plays an important role in fat mobilisation in the epithelium of the lumen. The occurence of lipase is an indirect indication of the capacity for utilisation of fat for energy source. Higher the activity of the lipase appears to hydrolyse the fat into fatty acids and glycerol. When required for other physiological activities, lipase activity found to be in high in larvae fed on sunflower and cotton as compared to tomato, Bhendi, red gram and Bengal gram.

The food containing high protein content, viz., Bengal gram, red gram and sunflower, strongly stimulate the protease activity as compared to tomato and Bhendi. Ishayya *et al.* (1970) also reported that proteolytic activity was strongly stimulated by diet with high protein content. Alibekova and Shvetsova (1991) indicated greater level of amylase and marginally protease activity and less level of invertase in the gut of *H.armigera* reared on resistant cotton varieties than those reared on susceptible varieties.

Ananthakrishnan *et al.* (1994) observed that the enzyme activity,in *H.armigera* varied with different varieties of cotton. They further reported that the amylase, invertase and protease enzyme were found to be more when *H.armigera* larva fed on boll of Suvin variety thant MCU7, TCHB 213 and MCU11. However, the activity of amylase, invertase, protease and lipase found to be more when *Spodoptera litura* larvae fed on the leaves of MCU11 followed by Suvin as compared to MCU7 and LRA 5166. In *Earis vittella*, the activity of amylase, invertase, protease and lipase found to be more on preferred host Suvin as compared to MCU7 and MCU11. Whereas the activity of these digestive enzymes found to be more when *Pectinophora gossypiella* larvae fed on the bolls of TCHB 213 than MCU11, MCU7, Suvin and LRA5166 (Ananthakrishnan, *et al.* 1994,).

From the data, it can be concluded that the type of food an insect consumes stimulates the digestive enzyme, probably through phagostimulant mechanism.

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Short Communication

Laboratory Evaluation of Diflubenzuron : Deliterious Effects on Ovaries of *Poekilocerus pictus* (Fabr.)

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Abstract: Diflubenzuron is an important member of newly synthesized series of benzoyl phenyl urea compounds. These compounds mainly effect synthesis of chitin. In addition to this peculiar mode of action author studied a number of side effects and it is noticed that diflubenzuron also cause serious damage to the ovary of the treated *P. pictus* (Fab.)

Keywords: Diflubenzuron, ovary, *P. pictus*

INTRODUCTION

Many reports are available (Hajjar and Casida, 1979; Mathur, 1995) indicating effect of benzoyl phenyl urea compounds on various insects. Mode of action of Diflubenzuron is almost fixed that is by inhibiting synthesis of chitin (Saxena and Mathur, 1980) but there is no report available regarding its side effect on *P. pictus*. In the present report its side effect on the ovary of *P. pictus* is discussed.

Diflubenzuron was dissolved in acetone and applied to 2 days old virgin female *P. pictus* on the ventral side of the abdomen at a dose level of 20 μg/insect. Controls were treated with acetone. These female were crossed with normal males. Each experiment had 4 pairs of adults and replicated 3 times.

The treated females were dissected after 15 days of treatment in physiological saline. Ovary was fixed in Bouin's fixative for 24 hours and then dehydrated in alcohol series, cleared in xylene and embedded in histowax (57° c). The sections were cut at a thickness of 5μ.

The results reveal that the abdomen in few treated females could not come out of sand after egg laying and mortality occurred in the same position. When the abdomen was stretched back, the normal position was not attained again. This may be attributed to chitin synthesis inhibiting activity of diflubenzuron.

The ovary of the treated females shows smaller ovarioles. A characteristic feature in the ovary of few treated females is the presence of black spot at the base of most of the ovarioles. Histologically it is observed that the cytoplasm of the oocyte breaks into fragments. All cellular materials degenerate and high vacuolization is observed.

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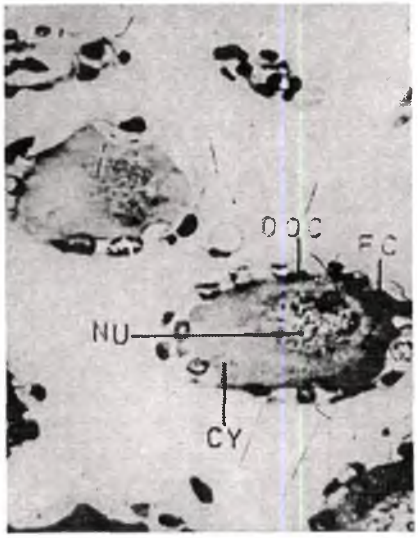

Fig. 1.

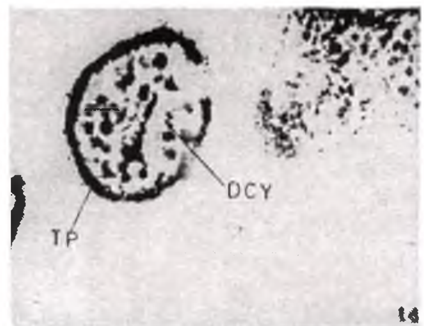

Fig. 2.

Fig. 1. Histomicrograph of ovary of control *P. pictus* x100
Fig. 2. Histomicrograph of ovary of *P pictus* treated with diflubenzuron showing destruction of oocyte. (15 days after treatment)x 100; abbreviations used: ooc = Oocyte; CY = Cytoplasm; TP = Tunica Propria; DCY = Degenerating cytoplasm; FC = Follicular cells; NU = Nucleus;

Few oocytes show pigmented material which corresponds to dark spots observed at the base of most of the ovarioles. Nuclei of most of the oocytes disintegrate and the lumen of the oviduct shows eosinophillic secretions (Figs. 1&2).

The fall in the reproducibility after treatment with diflubenzuron may be attributed to the adverse effect on the ovary of the treated females which is in accordance to Gregory and Kramer (1976) who reported suppressed progeny development in 6 Coleopteran species on applying dimilin and Hajjar and Casida (1979) who reported reduction in reproducibility in *O. fasciatus* treated with diflubenzuron.

Thus the results suggest that the adult stage is also suitable for the treatment since diflubenzuron has shown great potential for the suppression of reproduction in *P. pictus*.

ACKNOWLEDGEMENTS

Thanks are due to Dr. S. C. saxena, Professor, Department of Zoology, University, of Rajasthan, Jaipur under whose supervision this work was carried out.

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SHORT COMMUNICATION

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